The Journal of
CLINICAL
ENDOCRINOLOGY

VOLUME 11 NOVEMBER, 1951 NUMBER 11
Copyright 1951 by the Association for the Study of Internal Secretions

p-HYDROXYPROPIOPHENONE (PHP) AND OTHER SO-CALLED PITUITARY INHIBITORS*

C. A. SCHAFFENBURG, M.D., GEORGES M. MASSON, PH.D. AND E. PERRY McCULLAGH, M.D. (with the collaboration of PIERRE HAOUR, M.D., Hôpital Herriot, Lyon, France)

From the Research Division and Department of Endocrinology, Cleveland Clinic, and the Frank E. Bunts Educational Institute, Cleveland, Ohio

THERAPEUTIC inhibition of anterior pituitary activity by substances other than estrogens has long been a provocative if hitherto unproductive field of investigation. Such an action would be important in diseases due to pituitary hyperactivity. For this reason the recent claim by Perrault (1) in France, that pituitary inhibition is a property of a synthetic compound, *p*-hydroxypropiophenone (PHP), seemed worthy of critical study.

EXPERIMENTAL

I. The estrogenic activity of PHP was tested in 9 spayed female rats, each weighing about 250 Gm. The substance was first added to the diet (daily dose, 15 to 25 mg.), then administered in the form of 280-mg. pellets implanted subcutaneously, and finally given as a water suspension by stomach tube (200 mg. daily). All results were negative, judged by vaginal smears and uterine weights.

II. The antithyrotropic effects of PHP were tested in propylthiouracil-treated rats and in thyroidectomized rats. Criteria of activity were inhibition of gain in thyroid weight and absence of thyroidectomy cells in the

Received for publication July 16, 1951.

* This investigation was supported partly by a grant from Mr. Dean Francis and by a grant from the National Heart Institute, Public Health Service.

anterior pituitary. In the first experiment 30-day-old female rats were divided into two groups of 13 animals each and treated as follows: group I received propylthiouracil in a dose of 2.5 mg. per Gm. of food, and group II received propylthiouracil (2.5 mg.) plus PHP (1.0 mg.) per Gm. of food. The animals were sacrificed on the fifteenth day. Although thyroid weight was somewhat decreased in group II (mean, 20.5 mg.), in comparison with group I (mean, 25.2 mg.), this difference was not statistically significant.

In the second experiment two groups of 10 female rats, each weighing about 100 Gm., were thyroidectomized and PHP added to the food of one of these groups as in the previous experiment. On the twenty-fifth day the animals were sacrificed. Histologic study of the pituitary did not reveal any difference between the two groups.

III. Two groups of 24 female rats, 21 days old and 34 days old, respectively, were subdivided into four groups as follows: a) spayed, treated; b) spayed controls; c) intact, treated; d) intact controls. PHP was administered in the diet as in previous experiments. The animals were sacrificed at the end of twenty-five days. No significant change was noted in any of the organs studied (pituitary, ovaries, uterus, adrenals, thymus, thyroids and kidneys). Uterine weight was somewhat greater and ovarian weight was smaller in the treated animals than in their controls. There was no decrease in castration cells of the pituitary in the spayed, treated animals.

IV. Two groups of 24 male rats, 21 days old and 30 days old, respectively, were subdivided into four groups of 6 animals each as follows: a) castrate, treated; b) castrate controls; c) intact, treated; d) intact controls. The treated animals received PHP in the diet as previously, and also in the form of 200-mg. pellets implanted subcutaneously. The experiment was terminated at the end of four weeks and the organs were fixed, weighed and sectioned. Seminal vesicles and prostate were weighed fresh, and alkaline phosphatase determined in the latter organ by the method of Huggins and Talalay (2). The results are shown in Table 1.

There was a decrease in the weight of the pituitary of the treated animals, both castrate and intact, which was statistically significant ($P < 0.01$). In the intact, treated animals there was an inhibition of seminal vesicle and prostate weights and prostatic phosphatase content, which was significant only for the 21-day-old animals ($P < 0.01$) when compared with their respective controls. No significant effect was observed on testes or kidneys. There was an increase in adrenal weight (calculated per 100 grams of body weight) in the treated animals, with pronounced thymus involution. The weight of the thyroids "pooled" for both age groups, revealed an inhibition in the treated animals which was significant only for the intact rats ($P < 0.01$). Body growth was inhibited in all treated animals.

TABLE 1. EFFECT OF *p*-HYDROXYPROPIOPHENONE (PHP) IN CASTRATE AND IN INTACT MALE RATS

	Castrate rats				Intact rats			
	PHP		Controls		PHP		Controls	
	30-day-old	21-day-old	30-day-old	21-day-old	30-day-old	21-day-old	30-day-old	21-day-old
No. of rats	4	3	4	5	5	5	4	6
Initial body wt. (Gm.)	63.6 (58–69)*	43.3 (40–45)	58.0 (50–68)	42.0 (39–46)	72.4 (58–84)	48.4 (42–54)	74.0 (63–86)	47.0 (43–52)
Final body wt. (Gm.)	87.5 (80–104)	112.3 (102–132)	133.0 (123–149)	139.0 (120–155)	93.6 (75–107)	126.6 (96–150)	164.0 (123–188)	159.0 (136–179)
Seminal vesicles (mg.)	11.3 (6.2–23.0)	6.2 (5.6–7.0)	7.9 (6.0–10.0)	8.4 (8.0–8.6)	45.7 ±19.5†	66.6 ±11.4	182.5 ±89.8	221.5 ±22.1
Prostate (mg.)	25.3 (20.0–28.0)	19.4 (14.2–22.0)	25.2 (20.4–31.2)	25.4 (22.6–29.0)	105.7 ±30.9	144.6 ±11.2	212.4 ±52.2	266.3 ±29.6
Alk. p'tase, H. T. units/prost.	1.78 (1.36–2.16)	1.61 (1.06–2.0)	1.16 (0.96–1.36)	1.25 (1.08–1.44)	21.69 ±12.5	24.0 ± 1.31	34.32 ± 8.71	44.8 ± 7.02
Pituitary (mg.)	3.6 ± .31	—	5.5 ± .28	—	2.8 ± .12	—	4.5 ± .50	—
Testes (Gm.)	—	—	—	—	1.51 (0.50–2.60)	1.64 (1.35–2.01)	1.71 (0.61–2.60)	2.14 (2.0–2.4)
Thymus (Gm.)	0.074 (.046–.139)	0.203 (.195–.230)	0.318 (.267–.343)	0.339 (.264–.392)	0.096 (.061–.178)	0.223 (.131–.305)	0.320 (.250–.410)	0.292 (.182–.412)
Adrenals, mg./100 Gm. body wt.	21.9 (20.0–23.8)	21.8 (15.6–26.0)	18.0 (14.9–21.7)	20.2 (15.1–24.6)	20.3 (17.3–25.8)	17.5 (13.3–24.4)	13.9 (10.6–20.0)	14.0 (11.9–16.8)
Thyroid (mg.)	6.8 ± .64		8.4 ± .49		6.5 ± .36		10.7 ± .93	

* Figures in parentheses represent range.
† Figures preceded by ± sign represent standard error.

V. PHP was administered to a dog with hypertension of a month's duration following silk (wrapped kidneys) perinephritis and unilateral nephrectomy. The blood pressure was persistently elevated at a level of ±200 mm. of Hg. No change in blood pressure resulted from administration of PHP in oral doses up to 500 mg. daily for a month.

CLINICAL TRIALS

p-Hydroxypropiophenone was administered to 5 patients suffering from the following conditions: 2 with long-standing male prepuberal hypogonadism, 2 with Graves' disease, and 1 with acromegaly.

I. In the first 2 patients eunuchoidism had followed cryptorchidism and had been controlled for some years with testosterone. Their urinary gonadotropins ran consistently above 318 M.U. by the method of Klinefelter *et al.* (3) and both had a slight degree of gynecomastia. PHP was administered orally in 250-mg. capsules in doses ranging from 750 to 1,500 mg. daily for sixty-five and seventy days, respectively. Testosterone was discontinued during PHP administration in one patient while the other continued to receive testosterone in doses that were known by previous assays not to inhibit his gonadotropin excretion. In neither case did PHP cause a decrease in urinary gonadotropins. There were no subjective or objective changes noted.

II. The 2 women suffering from Graves' disease and the menopause had moderate exophthalmos (AP, in mm.: OD 23, OS 20; fissures: OD 12, OS 11, for one patient. AP: OD 21, OS 22; fissures: OD 12, OS 15, for the other). The BMR was normal in both patients at the time of treatment; one of them had received radioactive iodine prior to PHP administration. PHP was administered in doses of 750 to 2,000 mg. daily for sixty-eight to seventy-five days, respectively. There was no change in the degree of exophthalmos (AP, in mm.: OD 23, OS 20; fissures OD 11, OS 12. AP: OD 21, OS 22; fissures: OD 12, OS 15, respectively). The urinary gonadotropins remained elevated, above 318 M.U. In both cases there was some elevation of serum cholesterol, not considered significant.

III. The last patient was a 47-year-old woman suffering from acromegaly and hypertension of ten to eleven years' duration. She had been given estrogens, which lowered the level of the blood phosphorus, reduced the size of her hands and corrected the diabetic type of glucose tolerance curve. At the time of PHP administration she had not taken estrogen for three months. PHP was administered in doses ranging from 750 to 1,500 mg. per day for fifty-six days, in spite of which her condition became worse, the level of blood phosphorus remained high (5.2 mg.), there was no change in size of the hands, and arterial pressure remained elevated.

DISCUSSION

Inhibition of pituitary gonadotropins by large doses of estrogenic hormones is now a well known fact. This inhibition has been demonstrated by different means in animal experiments (4, 5, 6). In man, it was strikingly demonstrated by assay of pituitaries obtained post mortem from menopausal women treated with estrogens; the pituitaries showed great decrease in gonadotropin content (7). The question of dosage is important, since either small dosage or short-term administration of estrogens seems to stimulate LH release (8, 9).

Estrogen inhibition of other pituitary tropic hormones rests on somewhat less firm grounds. There is some evidence, both experimental (10) and clinical (11), that estrogens will inhibit growth hormone when given in adequate amounts. The inhibition of thyrotropin is subject to question and actual proof is still lacking (12). Beneficial results from the use of large doses of estrogens in the treatment of Graves' disease have been claimed (13). On the contrary, ACTH is stimulated by estrogen (14, 15).

Since true pituitary inhibition is obtained only with large doses, which result in undesirable estrogenic effects, the aim of therapeutic research in this field is the dissociation of estrogenic and pituitary-inhibiting activity. Attention has been centered for some time on certain lactone derivatives of estrone. Smith (16, 17) originally found a stimulation of gonadotropins, possibly due to an increase in LH release, after treatment of rats with Westerfeld's lactone. A similar compound prepared by Jacobsen (18) yielded the same results, whereas estrolic acid and its derivatives inhibited gonadotropin secretion in immature female rats.

Subsequently Bradbury (19), using Westerfeld's lactone in doses which were mildly estrogenic, found no significant change in the gonadotropin content of the pituitary of the immature rat. More recently Mortimore *et al.* (20) assayed 14 different steroids for pituitary-inhibiting properties, but estrololactone prepared by Jacobsen's method was the only active substance. Its estrogenic activity, moreover, was in excess of its pituitary-inhibiting activity.

Several steroids and lactone derivatives, prepared by Dr. D. A. Prins in this laboratory, have been assayed for pituitary-inhibiting properties in immature male and female rats, single or in parabiosis, or by actual assay of the injected animal's pituitary (21). The substances studied, with the maximum daily dose employed, are as follows:

Iso-andrololactone acetate	1 mg.
Etiocholanolactone acetate	1 mg.
Marrianolic acid	500 gamma
Marrianolic acid lactone	250 "
Estrololactone acetate (Westerfeld)	100 "

Estrololactone acetate (Jacobsen)	100	"
Alcohol derivative of Jacobsen's lactone	150	"
Alcohol derivative of Westerfeld's lactone	100	"
Alcohol derivative of marrianolic acid	153	"
16//17-Androstane 3β,16,17-triol	200	"
16//17-Δ 5-Androstene 3β,16,17-triol	100	"
16//17-Estratriene 3,16,17-triol	200	"
3β-Hydroxy-Δ5-etiobilienic acid	100	"

All the nonestrogenic lactones and steroid derivatives were also devoid of effect upon the pituitary. Jacobsen's estrololactone acetate was the only active substance but the pituitary-inhibiting properties in all cases paralleled estrogenic effects. In no case was there a stimulation of gonadotropic activity. It appears possible, therefore, that the apparent stimulation observed by Smith (17) and by Jacobsen (18) in female rats is attributable to the real, though scant, estrogenic activity of the compounds used. It is also apparent that these compounds inhibit gonadotropins only insofar as they are estrogenic.

In view of the discouraging, hitherto unpublished, results obtained with the lactones the reports on propiophenone appeared promising. This substance is related to diethylstilbestrol, in that it may be described as being half the stilbestrol molecule. Its estrogenic effect is extremely low (22). Initially, Perrault *et al.* (23) reported the successful treatment of a case of malignant chorionepithelioma with pulmonary metastases in a woman. The patient was still free of clinical and radiologic signs and symptoms eighteen months after the first report. These authors then extended their observations to different conditions in which pituitary hyperactivity seemed to be involved (1). They concluded that PHP: (a) inhibits thyrotropic hormone, since it decreased the exophthalmos of Graves' disease; (b) inhibits FSH and growth hormone; (c) probably stimulates LH; and (d) is inactive against ACTH.

Our observations fail to substantiate these conclusions. That there is no inhibition of FSH appears evident from the experiments in female and male rats, in which there was no decrease in uterine or testicular weight. The possibility of LH inhibition remains when one considers that there was a significant decrease in the weight of the sex accessories in the 21-day-old male rats; in this respect our results are at complete variance with the conclusions of Perrault. PHP also failed to decrease the elevated urinary gonadotropins in 4 of the cases reported herein.

Regarding the case of malignant chorionepithelioma reported by Perrault, it should be pointed out that spontaneous remissions of these tumors are known to occur.

With regard to the effect on growth, we are inclined to attribute the stunting of the treated animals to toxicity of the substance rather than inhibition of growth hormone, since adrenal weight per 100 grams of body weight increased and there was pronounced thymus involution. The failure of response in the case of acromegaly substantiates this view.

The effect on thyrotropic hormone is questionable. Though there was a decrease in thyroid weight among the treated animals in Experiment No. IV, the substance was inactive in the thyroidectomized and in the propylthiouracil-treated rats (Experiment No. II). It failed also to influence the degree of exophthalmos in the 2 cases reported. These results would be in agreement with recent findings by de Gennes *et al.* (13), who failed to obtain improvement of exophthalmos in 2 cases of Graves' disease. That the question of the action of PHP on thyrotropic hormone is by no means clear is further illustrated by the report of Soulairac *et al.* (24), who found an increase in thyroidectomy cells (castration cells) following administration of the substance.

At present it appears possible, in view of these contradictory reports, that the action of PHP on the pituitary may be explained simply on the basis of a hormonal "shift" due to the toxicity of the substance, such as occurs after an alarm reaction (25).

Other possible pituitary inhibitors tested have included aqueous testicular extracts (26) and, more recently, aqueous extracts of lithosperm (*Lithospermum ruderale*) (27, 28, 29). In our hands "inhibin" preparations did not affect gonadotropin excretion in eunuchoid patients and had no effect on the hypophysis of castrated rats (30). In the case of lithosperm, recent evidence (31) seems to indicate that inhibition of pituitary tropic hormones is exerted directly upon these substances, and not via the pituitary, since inactivation takes place *in vitro*.

SUMMARY

The synthetic compound *p*-hydroxypropiophenone (PHP) was assayed for pituitary-inhibiting properties, both in animals and in a small number of clinical cases of pituitary hyperactivity.

No significant inhibition of pituitary function was obtained except in one experiment with male rats in which some inhibition of thyroid and sex-accessory weight occurred after administration of large doses of the drug. This effect is interpreted as being the result of a pituitary "shift" in hormonal activity due to nonspecific toxicity of the compound.

It is concluded that there is yet no compound available in which pituitary inhibition has been successfully dissociated from estrogenic activity.

Acknowledgments

The cooperation of Dr. I. H. Page and Dr. A. C. Corcoran is gratefully acknowledged.

We are also indebted to Miss D. Tweed, Miss E. Davy and George Wilson for technical assistance.

Addendum

Since this paper was submitted for publication other reports on negative results with PHP have appeared. Guillemin (32), Paulsen *et al.* (33) and Money *et al.* (34), independently, found that PHP did not inhibit somatotropin, thyrotropin or gonadotropins in experimental animals. Debeyre and Rudich (35) found PHP ineffective in 2 cases of malignant chorionepithelioma.

REFERENCES

1. Perrault, M.: État actuel du H-365, para-oxy-propiophénone, frénateur hypophysaire de synthèse, *Presse méd.* **58:** 1010, 1950.
2. Huggins, C., and Talalay, P.: Sodium phenolphthalein phosphate as a substrate for phosphatase tests, *J. Biol. Chem.* **159:** 399, 1945.
3. Klinefelter, H. F., Jr.; Albright, F., and Griswold, G. C.: Experience with a quantitative test for normal or decreased amounts of follicle stimulating hormone in the urine in endocrinological diagnosis, *J. Clin. Endocrinol.* **3:** 529, 1943.
4. Meyer, R. K.; Leonard, S. L.; Hisaw, F. L., and Martin, S. J.: Effect of oestrin on gonad stimulating power of the hypophysis, *Proc. Soc. Exper. Biol. & Med.* **27:** 702, 1929–30.
5. Meyer, R. K., and Hertz, R.: The effect of oestrone on the secretion of the gonadotropic complex as evidenced in parabiotic rats, *Am. J. Physiol.* **120:** 232, 1937.
6. Meyer, R. K.; Biddulph, C., and Finerty, J. C.: Pituitary-gonad interaction in immature female parabiotic rats, *Endocrinology* **39:** 23, 1946.
7. Rowlands, I. W., and Sharpey-Schafer, E. P.: Effect of estradiol benzoate on the amount of gonadotrophin found in the pituitary gland and urine of postmenopausal women, *Brit. M. J.* **1:** 205, 1940.
8. Halpern, S. R., and D'Amour, F. E.: Studies on the gonad-hypophyseal complex in estrin-injected rats, *Am. J. Physiol.* **115:** 229, 1936.
9. Lane, C. E.: Some influences of oestrin on the hypophyseal-gonad complex of the immature female rat, *Am. J. Physiol.* **110:** 681, 1934–35.
10. Reece, R. P., and Leonard, S. L.: Further evidence for a mammogenic factor in the rat hypophysis, *Proc. Soc. Exper. Biol. & Med.* **42:** 200, 1939.
11. Kinsell, L. W.; Michaels, G. D.; Li, C. H., and Larsen, W. E.: Studies in growth. I. Interrelationship between pituitary growth factor and growth-promoting androgens in acromegaly and gigantism. II. Quantitative evaluation of bone and soft tissue growth in acromegaly and gigantism, *J. Clin. Endocrinol.* **8:** 1013, 1948.
12. Salter, W. T.: The chemistry and physiology of the thyroid hormone, in The Hormones, edited by Pincus, G., and Thimann, K. V., New York Academic Press, 1950, vol. 2, p. 216.
13. de Gennes, L.; Bricaire, H.; Benzecry, I., and Villiaumey, J.: Les syndromes de Basedow d'origine diencéphalo-hypophysaire, *Presse méd.* **59:** 41, 1951.
14. Selye, H.: Stress, Montreal, Canada, Acta Inc., 1950, pp. 300 and 333.
15. Tepperman, J.; Engel, F. L., and Long, C. N. H.: A review of adrenal cortical hypertrophy, *Endocrinology* **32:** 373, 1943.

16. SMITH, O. W.: The pituitary responses of mature male rats to an oxidative inactivation product of estrone, *Endocrinology* **35:** 146, 1944.
17. SMITH, O. W.: Further studies on pituitary responses to an oxidative inactivation product of estrone, *Proc. Soc. Exper. Biol. & Med.* **59:** 242, 1945.
18. JACOBSEN, R. P.: Steroid lactones. I. The oxidation of estrone acetate to the lactone acetate of estrolic acid, *J. Biol. Chem.* **171:** 61, 1949.
19. BRADBURY, J. T.: Ovarian influence on the response of the anterior pituitary to estrogens, *Endocrinology* **41:** 501, 1947.
20. MORTIMORE, G. E.; PAULSEN, C. A., and HELLER, C. G.: The effect of steroids and lactone derivatives on hypophyseal gonadotrophin content, *Endocrinology* **48:** 143, 1951.
21. MASSON, G.; SCHAFFENBURG, C., and SEGUIN, F.: Unpublished data.
22. LACASSAGNE, A.; CHAMORRO, A., and BUN-HOI, N. P.: Sur quelques actions biologiques de la 4-hydroxy-propiophénone, *Compt. rend. Soc. biol.* **144:** 95, 1950.
23. PERRAULT, M.; VIGNALOU, J., and ETIENNE, M.: Chorionépithéliome malin (métastases pulmonaires actives) traité avec des résultats satisfaisants, cliniques, hormonaux et radiologiques, par le seul emploi d'un "frénateur hypophysaire de synthèse," la para-oxypropiophénone (corps H-365) pendant six mois, *Bull. et mém. Soc. méd. d. hôp. de Paris* **65:** 1008, 1949.
24. SOULAIRAC, A.; DESCLAUX, P.; CHANIAC, M., and TEYSSEYRE: Action endocriniene du *p*-oxypropiophénone. Reported at the Societé d'Endocrinologie de Paris, Jan. 25, 1951. *Presse méd.* **59:** 275, 1951.
25. SELYE, H.: The general adaptation syndrome and the diseases of adaptation, *J. Clin. Endocrinol.* **6:** 117, 1946.
26. MCCULLAGH, D. R.: Dual endocrine activity of the testis, *Science* **76:** 19, 1932.
27. DRASHER, M. L.: The mechanism of action of *Lythospermum ruderale, Endocrinology* **45:** 120, 1949.
28. NOBLE, R. L.; PLUNKETT, E. R., and TAYLOR, N. B. G.: Factors affecting the control of the pituitary gland, *Recent Progr. Hormone Research* **5:** 263, 1950.
29. PLUNKETT, E. R.; COLPITTS, R. V., and NOBLE, R. L.: Effect of *Lithospermum ruderale* on oestrus cycle of the rat, *Proc. Soc. Exper. Biol. & Med.* **73:** 311, 1950.
30. MCCULLAGH, E. P., and SCHAFFENBURG, C.: Unpublished data.
31. NOBLE, R. L.; PLUNKETT, E. R., and GRAHAM, R. C.: Direct inactivation of gonadotrophin, thyrotrophin and prolactin by extracts of *Lithospermum ruderale, Fed. Proc.* **10:** 97, 1951.
32. GUILLEMIN, R.: Parahydroxypropiophénone (H-365). Frénateur hypophysaire de synthèse? *Presse méd.* **59:** 799, 1951.
33. PAULSEN, C. A.; MORTIMORE, G. E., AND HELLER, C. G.: The pituitary action and estrogenic effect of parahydroxypropiophenone, *J. Clin. Endocrinol.* **11:** 892, 1951.
34. MONEY, W. L.; LUCAS, V.; FAGER, J., and RAWSON, R. W.: The effects of *p*-hydroxypropiophenone on the thyroid gland of experimental animals. *J. Clin. Endocrinol.* **11:** 973, 1951.
35. DEBEYRE, J., and RUDICH, E.: Deux cas de tumeur génitale à type de chorionépithéliome avec échec du H-365. Reported at the Societé de Gynécologie et d'Obstétrique de Paris, March 5, 1951. *Presse méd.* **59:** 1086, 1951.

FURTHER OBSERVATIONS ON THE URINARY EXCRETION OF WATER-SOLUBLE CORTICOSTEROIDS BY NORMAL AND ABNORMAL SUBJECTS*

N. B. TALBOT, M.D., M. S. WOOD, A.B., J. WORCESTER, D.P.H., E. CHRISTO, A.B., A. M. CAMPBELL, B.Sc. AND A. S. ZYGMUNTOWICZ, A.B.

From the Burnham Memorial Hospital for Children at the Massachusetts General Hospital, the Department of Pediatrics, Harvard Medical School and the Department of Biostatistics, Harvard School of Public Health, Boston, Massachusetts

INTRODUCTION

IN PREVIOUS papers, 300 preliminary observations on the urinary excretion of water-soluble corticosteroids by sick and healthy individuals have been reported (1, 2). The present communication records an additional approximately 350 measurements and summarizes studies carried out to date. The data presented indicate that this measurement serves as a reasonably reliable index of relative rates of adrenocortical 11-17-oxycorticosteroid production.

METHODS

Urines were collected without preservative and stored in the refrigerator. They were extracted and assayed according to a procedure described previously (3), which involves chloroform extraction of fresh urine adjusted to pH 6.5 and extensive purification of the extracts by alkali washing, partitioning between benzene and water and ketonic separation with Girard's reagent. The final measurement is based upon a copper-reduction type of assay, as used for the determination of sugar. Because this procedure appears to eliminate from the urine reducing agents other than water-soluble corticosteroids, it is apt, especially in normal and hypoadrenocortical subjects, to yield lower absolute values than are obtained by laboratories employing methods in which the urine extracts are subjected to less extensive purification. In this connection, studies reported by Tompsett and Oastler suggest that the step involving benzene-water partition is of relatively little importance and that it can be eliminated without altering results to a major extent (4).

In certain instances, data are recorded in terms of absolute milligrams

Received for publication May 23, 1951.

* This work was supported by the Commonwealth Fund of New York and the American Cancer Society.

of corticosteroids excreted per day. In other instances values obtained have been expressed in terms of milligrams per square meter of body surface (M^2) per day. Under this circumstance, the surface area of each individual was calculated from body weight or from body weight and height as described by Crawford (5).

RESULTS AND COMMENTS

The data obtained for normal and for abnormal individuals will be considered separately.

1. Normal subjects

Figure 1 gives information concerning the output of corticosteroids by normal persons of various ages. These persons were neither under stress nor recovering from a recent illness during the period of urine collection. In the upper half of the figure (Section A), absolute values per day are shown; in the lower half (Section B), the same data are expressed as milligrams per square meter (M^2) of body surface per day.

The data of Section A reveal that infants and small children excrete a lesser absolute quantity of corticosteroids than do older children and adults. Section B indicates, however, that with the possible exception of newborn infants the corticosteroid output per M^2 per day is nearly the same for individuals of all ages. In this series, the mean of 22 values obtained for 18 individuals under 7 years was 0.101, that of 30 values for 26 persons 7 to 14 years was 0.126, and the 78 values found for 31 subjects 15 or more years old was 0.143 mg. per M^2 per day.

Figure 2 describes the distribution of normal values shown in Figure 1 B. The pattern shown indicates a normal distribution above and below the mean value of 0.132 mg. per M^2 per day and describes a total range of from about .015 to .255 mg. per M^2 per day. The curve running upwards from left to right indicates what percentage of all the measurements (right-hand ordinate) fell at or below the values shown along the abscissa. As given in Table 1, the standard deviation from the normal mean value was $\pm .058$ mg. per M^2 per day.

Comments regarding normal subjects: The observation that the excretion of corticosteroids in the urine when adjusted for body size is essentially the same in all healthy persons has been confirmed by Read, Venning and Ripstein (6) and Norwal and King (7). This finding has two implications. First, it suggests that the basic needs of the healthy organism are proportional to size and that they are not greatly influenced by maturation during childhood and adolescence. This is not surprising in view of the apparent fundamental importance of this type of hormone in the bodily economy of individuals of all ages. It is, however, in sharp contrast to the

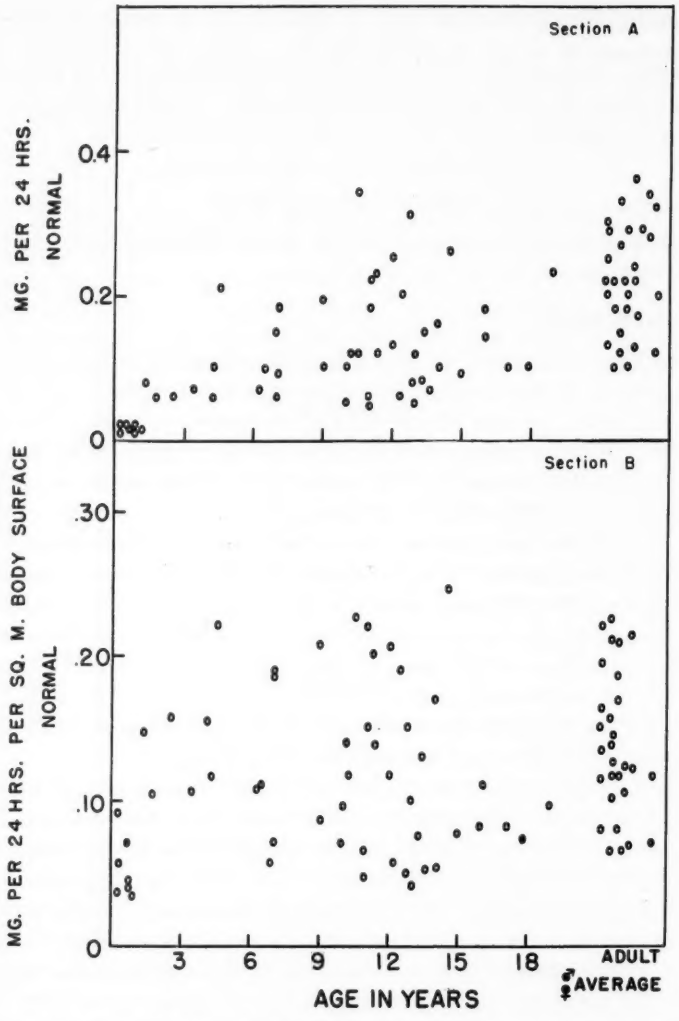


FIG. 1. Urinary output of corticosteroids by normal persons of various ages. Note that in Section A, the results are expressed as mg. per day and that in Section B they are expressed as mg. per M^2 of body surface per day.

well known fact that urinary 17-ketosteroids, which are generally thought also to be largely of adrenocortical origin, are eliminated at a much greater rate per M^2 per day in adolescents and adults than in infants and children (2, 8).

Second, it would appear that a single normal standard with respect to

basal urinary corticosteroid excretion can be used for all except newly born infants, without regard for age or sex. This permits easy comparison of values obtained on patients with various conditions whether they be older infants, children, adolescents or adults.

In the foregoing connection, it should be emphasized that normal standards for corticosteroid output, like normal standards for the basal metabolic rate, were obtained on persons thought to be healthy and to be in an approximately basal, or stress-free state with regard to adrenocortical activity. A variety of factors acting through physiologic mechanisms may

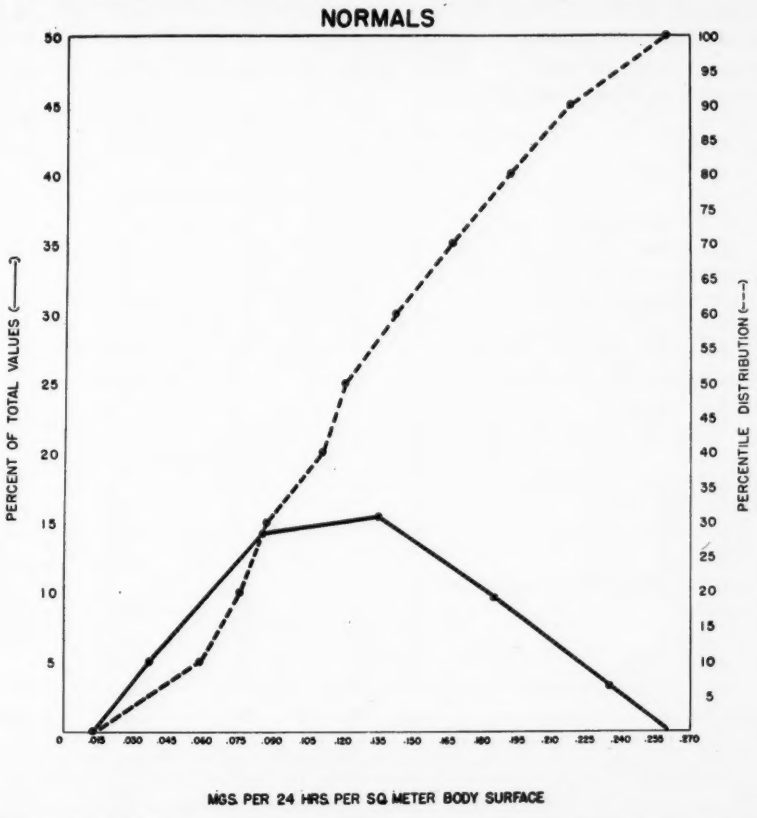


FIG. 2. Distribution of normal values shown in Figure 1B. The interrupted curve which courses upward from left to right should be read against the right-hand ordinate. It shows what portion of all the values were equal to or less than the values indicated by the scale along the abscissa. The solid curve, which should be read against the left-hand ordinate, gives the incidence of values falling approximately at the points indicated. Note that the scale for urinary corticosteroids along the abscissa is in mg. per M^2 per day.

TABLE 1. OBSERVATIONS ON THE URINARY CORTICOSTEROID EXCRETION BY PERSONS WITH VARIOUS CONDITIONS

Diagnosis	Number of subjects	Number of observations	Urinary corticosteroids (mg./M²/24 hrs.)	
			Mean	Standard deviation of distribution
		A. Low values		
Hypopituitarism	15	47	.054	.024
Diabetes mellitus, controlled	4	5	.058	—
Hypothyroidism, untreated	8	11	.062	.025
Hypoadrenocorticism (Addison's disease)	23	35	.079	.053
Osteoporosis	6	14	.089	.032
		B. Normal range		
Hypertension, essential	7	8	.091	—
Normal subjects	65	130	.132	.058
Ovarian agenesis	3	9	.134	—
Rheumatic fever, quiescent	12	15	.145	.073
Hirsutism, simple, nonendocrine	10	10	.147	.044
Hypothyroidism under treatment	5	9	.150	—
Hypogonadism (eunuchoidism)	5	7	.152	—
Allergy	5	5	.154	—
		C. High values		
Cushing's syndrome, inactive	12	58	.161	.079
Cold and starvation	2	7	.182	—
Obesity, simple	7	7	.182	—
Afebrile illness	3	6	.199	—
Adrenal cortical virilism due to hyperplasia	14	37	.202	.079
Acromegaly	10	22	.217	.138
Anorexia nervosa	4	8	.224	—
Hyperthyroidism untreated	7	7	.262	—
Febrile illness	20	22	.346	.179
Adrenal cortical virilism with Addisonian symptoms	3	8	.376	—
Burn	4	27	.440	.219
Cushing's syndrome, active	16	64	.683	.503
Adrenal cortical cancer	9	15	1.518	1.183
Cushing's syndrome, after stress of operation	4	13	1.649	.879

induce an increase in adrenocortical activity above its basal rate (9). It follows that healthy persons should be expected to have corticosteroid values falling within the standard normal range only under stressless circumstances.

2. Patients with various conditions

Figure 3 presents 640 measurements obtained on 315 patients falling into one of 38 diagnostic categories of which 27 are mentioned in Table 1. For reasons suggested previously, all results are expressed in terms of mg. per M^2 per twenty-four hours. The data set forth are divided into groups of low, normal and high values with reference to the data obtained on nor-

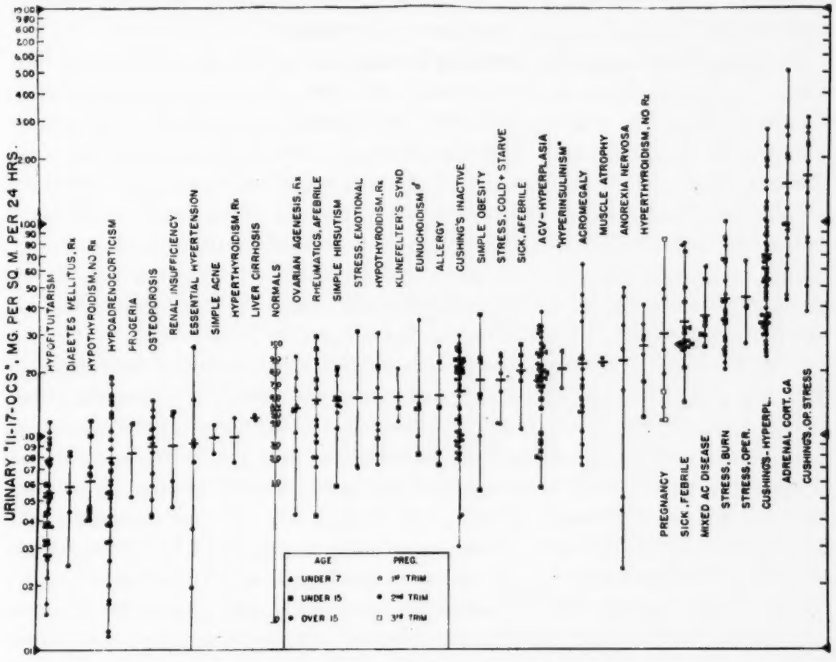


FIG. 3. Observations on the corticosteroid output of patients with various conditions. Each plot represents an individual value according to the logarithmic scale shown along the left-hand ordinate. The shape of the symbol gives the status of the individual with respect to age or phase of pregnancy, as indicated in the insert near the bottom of the figure. The total span of the vertical lines corresponding to each diagnosis gives the total range of the values obtained for patients with that diagnosis. The short, thick horizontal line crossing each vertical line indicates the average of the values shown along the latter line. In the case of the line representing normal persons, the numbers shown immediately above the short, thin horizontal lines correspond to the respective percentile points found along the interrupted curve of Figure 2.

mal persons. Mean values are given for each diagnostic group. Standard deviations of the distributions are given for those groups which contain 10 or more observations. The classification into low, normal or high was made by comparing the difference between the mean value of each group and that of the normals with the standard error of the difference between the mean values. The standard errors were computed from the standard deviations of the distribution for these groups when there were 10 or more observations; when there were less than 10 observations, the standard deviation of the normal individuals was used. When the mean value obtained was either below or above the normal by more than twice the standard error, the diagnosis involved was listed in the respective low or high category. The following remarks may be made concerning the groups found to have low, normal or high corticosteroid values.

a) **Comments regarding patients having low corticosteroid values.** The mean corticosteroid value for patients with primary hypoadrenocorticism (Addison's disease) was significantly low, though not so low as the values obtained for patients with hypopituitarism and with hypothyroidism. As shown in Figure 3, although most of the individual measurements obtained on the hypoadrenocortical patients were grossly subnormal, some were well within normal limits. Other workers, using different methods of assay, also have found that although most Addisonian patients show subnormal urinary corticosteroid values, occasionally they excrete approximately normal amounts of corticosteroid in the urine (10, 11).

It is believed that the occasional normal values reported here for Addisonian patients are valid and not the result of errors of overestimation. If this thesis is correct, it may be inferred that some hypoadrenocortical patients are suffering from partial rather than total limitation in ability to produce corticosteroids. This partial limitation would appear to be such that the diseased adrenal cortices, working at or near maximum capacity in response to ACTH stimulation, can elaborate normal basal quantities of corticosteroids, but cannot secrete the larger quantities which individuals with normal cortices ordinarily produce under conditions of stress in response to increased ACTH stimulation. This may explain the clinical observations, a) that desoxycorticosterone acetate alone constitutes effective replacement therapy for many patients with Addison's disease during periods when they are free from strain and, b) that such patients are apt to need 11-17-oxycorticosteroid (*i.e.*, cortisone or adrenal cortical extract) therapy in addition to desoxycorticosterone when they are subject to stress (12).

As mentioned previously, slightly lower mean corticosteroid values were obtained in patients suffering from hypopituitarism or from hypothyroidism than was the case in hypoadrenocortical subjects. On the basis of this

finding, one might expect patients with hypopituitarism or with hypothyroidism to have at least as many manifestations of adrenocortical insufficiency as patients with primary hypoadrenocorticism. This expectation, however, is not upheld by clinical experience. In contrast to the findings in the average patient with primary hypoadrenocorticism, in hypopituitarism and in hypothyroidism the patients usually do not manifest signs of adrenocortical insufficiency such as dehydration, hypotension, weakness and hypoglycemia (10, 13). The production of adrenocortical hormones in hypopituitarism and in hypothyroidism, though low when compared with the average normal figure, would therefore seem to be approximately adequate for ordinary bodily needs. If this thesis is correct, it follows that pituitary and thyroid insufficiency may result in a decrease in basal adrenocortical hormone requirements.

The tendency to low urinary corticosteroid values in the small series of patients with reasonably well controlled diabetes mellitus and in the patients with idiopathic osteoporosis, was unexpected. Other investigators have obtained normal values in well regulated diabetics (4, 10) and grossly elevated values in patients suffering diabetic coma (14). In the case of patients with osteoporosis the mean corticosteroid value falls only slightly below the normal range. On this account, it appears to be of doubtful physiologic or clinical significance.

b) **Comments regarding patients having corticosteroid values within the normal range.** The observation that a small group of patients with essential hypertension had a low normal mean corticosteroid value is of interest with relation to the possible role of the adrenal cortices in the causation of this condition. It suggests strongly that the hypertension is not due to hyperproduction of corticosteroids of the type responsible for Cushing's syndrome. On the other hand, this finding does not rule out the possibility that the adrenal cortices of certain patients with essential hypertension are elaborating excessive quantities of other types of adrenocortical hormone. For example, Locke *et al.* (15) have observed that 2 out of 4 patients with essential hypertension had sweat-chloride rate index values suggestive of excess production of a hormone resembling the desoxycorticosterone or sodium-potassium metabolism type. The values for patients with ovarian agenesis were well within normal limits, which is in contrast to observations which indicate that the urinary 17-ketosteroid output of such patients tends to be somewhat lower than normal (16). This divergence in results obtained by separate indices of adrenocortical function is similar to that seen in normal individuals of various ages (2).

Normal mean corticosteroid values also were obtained in patients having quiescent rheumatic fever, simple hirsutism, treated hypothyroidism, eunuchoidism or allergy.

In the case of patients with allergy (asthma, eczema) it is of interest, in view of their tendency to be benefited at least temporarily by ACTH and cortisone therapy (17), to note the high normal mean corticosteroid value. This finding would appear to militate against the possibility that this type of patient is suffering from absolute hypoadrenocorticism.

c) **Comments regarding patients having high corticosteroid values.** To facilitate discussion, the conditions included under this heading in section C of Table 1 will be considered under the following somewhat arbitrary subheadings: 1) pathologic hyperadrenocorticism, 2) physiologic hyperadrenocorticism, and 3) miscellaneous.

1) *Pathologic hyperadrenocorticism.* This heading includes patients with signs either of Cushing's syndrome or of virilism. In the present group, causes of virilism other than hyperadrenocorticism have been excluded by appropriate procedures. Patients with hyperadrenocorticism and signs of Cushing's syndrome or virilism or both, due to adrenocortical cancer, have been listed separately under the latter diagnosis. The remaining patients were thought almost certainly to have adrenocortical hyperplasia.[1] It is seen that, with the possible exception of patients with apparently inactive Cushing's syndrome, all patients falling into this group had abnormally high corticosteroid values.

The mean value obtained for patients with adrenocortical virilism was only moderately elevated above the normal range. This is in contrast to their urinary 17-ketosteroid excretion levels which were elevated to the high levels characteristic of patients with this disease (16, 18). Patients having, in addition to virilism, disturbances in electrolyte and water balance similar to those seen in patients with hypoadrenocorticism (19), had a mean corticosteroid value which was almost twice as great as that found for patients with simple virilism. The mean value for individuals with active Cushing's syndrome (0.683 mg. per M^2 per day) was over three times as high as that for patients with simple adrenocortical virilism (0.202 mg. per M^2 per day). The mean value obtained for patients with adrenocortical cancer (1.518 mg. per M^2 per day) was considerably higher than those mentioned above. However, as shown in Figure 3, individual values obtained on patients with hyperadrenocorticism due to hyperplasia overlapped those found for subjects with cancer of the adrenal cortex. This observation indicates that random urinary corticosteroid measurements cannot be used as a means for distinguishing between these two types of adrenocortical pathology. These findings are in general agreement with those of others (4, 10, 11, 20, 21).

---

[1] This diagnosis was based in some instances upon the results of biopsy of adrenal tissue. In others it was based on the fact that the patient had signs of congenital adrenocortical virilism.

The highest mean corticosteroid output occurred in patients with Cushing's syndrome due to bilateral adrenocortical hyperplasia, in periods immediately following operative exploration of the adrenal glands. Presumably, the increase in output over that seen in patients with this condition who were not operated on, is attributable to the nonspecific stress induced by anesthesia and surgery. This is of interest, for it suggests that patients with Cushing's syndrome, like normal individuals, are capable of homeostatic variations in the activity of their anterior pituitary-adrenocortical system.

2) *Physiologic hyperadrenocorticism.* Included under this heading are individuals suffering either stress due to cold plus starvation, miscellaneous afebrile or febrile illnesses, anorexia nervosa, hyperthyroidism or extensive thermal burn.

Those exposed to cold and starvation were healthy, young male volunteers. They were adequately clothed but lived in a room, the temperature of which was maintained at −20°F. They were given one liter of water, but no food during the six-day period of study. The mean value for this total period was only slightly elevated above the normal mean. The individual values which make up this mean underwent a slight rise above control values in one subject on Day 1, and in the other subject on Day 3 of the experimental regimen. Thereafter, values tended to return to control levels. Control values for the 2 subjects were .13 and .19 mg. and maximum values were .24 and .24 mg. per M^2 per day, respectively. Urinary 17-ketosteroid values determined simultaneously decreased 50 and 75 per cent from respective control values of 14.5 and 23.5 mg. per day during the six days of study. These observations suggest that this type of stress induces only a minor change in corticosteroid metabolism and, paradoxically, a considerable change in 17-ketosteroid metabolism. Moreover, the absence of any large change in corticosteroid output under conditions of fasting (1) and of fasting plus exposure to cold is noteworthy in view of the known actions of 11-17-oxycorticosteroids upon carbohydrate metabolism. By facilitating gluconeogenesis from protein and by inhibiting tissue carbohydrate utilization, this type of adrenocortical hormone tends to counteract the tendency to hypoglycemia inherent in subjects undergoing prolonged fasting (22). Apparently the body is able to prevent fasting hypoglycemia without augmenting corticosteroid output to a major extent.

The rise in mean corticosteroid values observed in patients suffering the stress of miscellaneous afebrile illnesses and burns is in keeping with the alarm reaction thesis of Selye (9) and with the reports of other workers (4, 14, 23, 24). Quantitatively speaking, it is of considerable interest to note that the mean value found for burn and surgical patients corresponds closely to that recorded for patients with active Cushing's syndrome due to bilateral adrenocortical hyperplasia.

This finding indicates that there is not necessarily any absolute difference between the adrenocortical activity of endocrinologically normal persons on the one hand and patients with pathologic hyperadrenocorticism on the other. It would, therefore, appear that the basic difference between physiologic and pathologic hyperadrenocorticism resides in relations between rates of adrenocortical hormone production and the physiologic needs of the body for these hormones, rather than in differences in degree of adrenocortical activity. In physiologic hyperadrenocorticism, hormone production is in approximate accordance with the body's requirements. Pathologic hyperproduction of corticosteroids leads to the changes seen in Cushing's syndrome, whether spontaneous or as a result of ACTH treatment. By contrast, physiologic hyperadrenocorticism ordinarily fails to induce these changes even when it persists, as in certain burn patients in whom hypercorticosteroiduria lasts for as long as fifty to sixty days (1).

d) **Remarks on miscellaneous other observations.** Figure 3 contains limited observations on patients with renal insufficiency, simple acne, treated thyrotoxicosis, cirrhosis of the liver, emotional stress, Klinefelter's syndrome, hypoglycemia due to "hyperinsulinism," and muscular atrophy, respectively. In none of these conditions were the values grossly abnormal.

SUMMARY AND CONCLUSION

The urinary corticosteroid output of healthy, unstressed individuals expressed as milligrams per square meter of body surface per day is shown to be essentially the same at all ages (average 0.132, standard deviation 0.058 mg. per M^2 per day). Extension of this method of expressing measurements to patients with various conditions permits easy comparison of results obtained on individuals of different sizes suffering from the same disease.

The previously noted tendency of hypoadrenocortical, hypopituitary and hypothyroid patients to have a subnormal corticosteroid output has been confirmed. In addition, suggestively low mean values were obtained for a small group of patients with controlled diabetes mellitus. The mean corticosteroid excretion of patients with idiopathic osteoporosis was slightly below normal limits, a finding of doubtful significance.

The mean corticosteroid values of patients with essential hypertension, ovarian agenesis, quiescent rheumatic fever, simple nonendocrine hirsutism, adequately treated hypothyroidism, eunuchoidism, and allergy, respectively, fell within the normal range.

Abnormally high mean corticosteroid values were obtained in patients with pathologic hyperadrenocorticism due to adrenocortical cancer, as evidenced clinically by signs of virilism or of Cushing's syndrome. Patients with bilateral adrenocortical hyperplasia who showed only signs of

virilism had a slightly to moderately elevated mean value of about 0.2, whereas those who displayed manifestations of Cushing's syndrome had a mean value which was markedly elevated to about 0.7 mg. per M^2 per day. There was, however, considerable overlap between the values found for individuals with adrenocortical hyperplasia as compared to adrenocortical cancer. Hence it is concluded that measurement of corticosteroids cannot *per se* be relied upon to distinguish between these two forms of adrenocortical pathology.

Patients with physiologic hyperadrenocorticism occasioned by the stress of a variety of conditions including febrile illnesses, operation and burn, excreted urinary corticosteroid at rates comparable to those observed in patients with pathologic hyperadrenocorticism.

To date, over 1,400 urinary corticosteroid measurements have been performed in this laboratory. The data thus obtained support the conclusion that this determination may be considered a reasonably reliable index of adrenal corticosteroid production.

REFERENCES

1. Talbot, N. B.; Albright, F.; Saltzman, A. H.; Zygmuntowicz, A., and Wixom R.: The excretion of 11-oxycorticosteroid-like substances by normal and abnormal subjects, *J. Clin. Endocrinol.* **7:** 331, 1947.
2. Talbot, N. B.: Physiology of the adrenal cortex, *Pediatrics* **3:** 515, 1949.
3. Talbot, N. B.; Saltzman, A. H.; Wixom, R. L., and Wolfe, J. K.: Colorimetric assay of urinary corticosteroid-like substances, *J. Biol. Chem.* **160:** 535, 1945.
4. Tompsett, S. L., and Oastler, E. G.: The excretion of corticosteroids: the determination of the total free reducing ketosteroids in urine, *Glasgow M. J.* **28:** 349, 1947.
5. Crawford, J. D.; Terry, M. E., and Rourke, M.: Simplification of drug dosage calculated by application of the surface area principle, *Pediatrics* **5:** 783, 1950.
6. Read, C. H.; Venning, E. H., and Ripstein, M. P.: Adrenal cortical function in newly-born infants, *J. Clin. Endocrinol.* **10:** 845, 1950.
7. Norval, M. A., and King, N. A.: A biometric study of the excretion of corticosteroids in children in relation to age, height and weight, *Biometrics*, **6:** 395, 1950.
8. Day, E. M. A.: The urinary excretion of 17-ketosteroids and of corticosteroid-like hormones by the newborn infant, *M. J. Australia* **2:** 122, 1948.
9. Selye, H.: The general adaptation syndrome and the diseases of adaptation, *J. Clin. Endocrinol.* **6:** 117, 1946.
10. Venning, E. H., and Browne, J. S. L.: Excretion of glycogenic corticoids and of 17-ketosteroids in various endocrine and other disorders, *J. Clin. Endocrinol.* **7:** 79, 1947.
11. Daughaday, W. H.; Jaffe, H., and Williams, R. H.: Adrenal cortical hormone excretion in endocrine and nonendocrine disease as measured by chemical assay, *J. Clin. Endocrinol.* **8:** 244, 1948.
12. Thorn, G. W.; Forsham, P. H., and Emerson, K., Jr.: The Diagnosis and Treatment of Adrenal Insufficiency; a monograph in American Lectures in Endocrinology, edited by W. O. Thompson. Springfield, Illinois, Charles C Thomas, Publisher, 1949.

13. Talbot, N. B.; Sobel, E.; McArthur, J., and Crawford, J. D.: Functional Endocrinology from Birth through Adolescence. The Commonwealth Fund of New York (in press).
14. McArthur, J. W.; Sprague, R. G., and Mason, H. L.: The urinary excretion of corticosteroids in diabetic acidosis, *J. Clin. Endocrinol.* **10:** 307, 1950.
15. Locke, W., and Talbot, N. B.: The electrolyte composition of sweat in relation to adrenal cortical function, *J. Clin. Investigation* **30:** 325, 1951.
16. Fraser, R. W.; Forbes, A. P.; Albright, F.; Sulkowitch, H., and Reifenstein, E. C., Jr.: Colorimetric assay of 17-ketosteroids in urine, *J. Clin. Endocrinol.* **1:** 234, 1941.
17. Thorn, G. W.; Forsham, P. H.; Frawley, T. F.; Hill, S. R., Jr.; Roche, M.; Staehelen, D., and Wilson, D. L., Medical progress: the clinical usefulness of ACTH and cortisone, *New England J. Med.* **242:** 783, 824, 865, 1950.
18. Talbot, N. B.; Butler, A. M., and Berman, R. A.: Adrenal cortical hyperplasia with virilism: diagnosis, course and treatment, *J. Clin. Investigation* **21:** 559, 1942.
19. Gardner, L. I.; Zygmuntowicz, A. S.; Sniffen, R., and Talbot, N. B.: Follow-up studies in a boy with mixed adrenal cortical disease, *Pediatrics* **5:** 808, 1950.
20. Lowenstein, B. E.: Corcoran, A. C., and Page, I. H.: Determination of corticosteroids in urine, (abstract) *J. Clin. Endocrinol.* **6:** 481, 1946.
21. Sprague, R. G.; Hayles, A. B.; Power, M. H.; Mason, H. L., and Bennett, W. A.: "Steroid diabetes" and alkalosis associated with Cushing's syndrome: report of a case, isolation of 17-hydroxycorticosterone (compound F) from urine, and metabolic studies, *J. Clin. Endocrinol.* **10:** 289, 1950.
22. Thorn, G. W.; Koepf, G. F.; Lewis, R. A., and Olsen, E. F.: Carbohydrate metabolism in Addison's disease, *J. Clin. Investigation* **19:** 813, 1940.
23. Weil, P. G., and Browne, J. S. L.: The excretion of cortin under conditions of damage, *J. Clin. Investigation* **19:** 772, 1940.
24. Shipley, R. A.; Dorfman, R. I.; Buchwald, E., and Ross, E.: The effect of infection and trauma on the excretion of urinary cortin, *J. Clin. Investigation* **25:** 673, 1946.

THYROID-THYROTROPIC HORMONE BALANCE IN THE BLOOD OF NORMAL AND ENDOCRINOPATHIC INDIVIDUALS*†

SAVINO A. D'ANGELO, PH.D.,** KARL E. PASCHKIS, M.D.,‡
ALBERT S. GORDON, PH.D. AND
ABRAHAM CANTAROW, M.D.§

From the Division of Endocrine and Cancer Research, Jefferson Medical College, Philadelphia, Pa., and the Department of Biology, Washington Square College, New York University, New York, N. Y.

IN VIEW of the well established, fundamental nature of the thyroid-pituitary relationship in the mammalian organism (1), it has often been suspected that altered thyroid gland function in certain disease states is referable to, or may subsequently effect, change in the thyrotropic mechanism of the anterior hypophysis (2, 3). Although the status of thyroid function in the patient has been frequently and reliably measured by the collective use of such criteria as the basal metabolic rate, the protein-bound iodine (serum-precipitable, "hormonal" iodine), and the radioiodine uptake (4, 5, 6), precise evaluation of thyroid-stimulating hormone (TSH) activity has presented greater difficulty. Some information regarding the role of TSH in regulating thyroid function in normal and in endocrine patients has been obtained from studies utilizing exogenous thyrotropin. Basal metabolic rate and blood hormonal iodine levels rise after TSH administration (7), as does the rate of radioiodine uptake (8). Exogenous TSH, in conjunction with tracer techniques, also enables differentiation of primary hypothyroidism from that secondary to anterior pituitary failure (6, 9). Accurate measurement of the endogenous thyrotropic hormone activity in the patient, however, has not been accomplished. In this regard, two major factors appear to be responsible: 1) neither the cytologic picture nor the TSH content of the human pituitary can be determined clinically, and 2) TSH levels in body fluids are so low as to require for their detection methods of unusual sensitivity. Despite the latter limitation, evidence has accumulated to indicate that endogenous TSH blood levels vary unduly in endocrinopathy (10, 11, 12).

Received for publication June 18, 1951.

* Read at the Annual Meeting of the Association for the Study of Internal Secretions, Atlantic City, N. J., June 8, 1951.

† Supported, in part, by a grant (RG-2407) from the National Institutes of Health.

** Dept. of Anatomy.

‡ Depts. of Medicine and Physiology.

§ Dept. of Biochemistry.

It is generally recognized that accurate appraisal of the thyroid-pituitary axis in health and disease must involve measurement of at least two prime variables in the hormonal balance (2, 3). Rarely, however, have both thyroid and TSH levels been determined in the blood of the same individual. Recent experiments by D'Angelo and Gordon (13) have shown that thyroid and thyrotropic hormone activity can be detected simultaneously in small samples of untreated serum with the stasis tadpole technique of bioassay, and tentative definition of the blood "thyroid-TSH" balance in normal, experimental, and clinical conditions has been given with the method (12, 13, 14). The present report is concerned with a clinical study in which the blood thyroid-TSH equilibrium has been determined in normal individuals and in groups of patients with known or suspected pituitary or thyroid dysfunction (acromegaly, Simmonds' disease, hypothyroidism, hyperthyroidism, and ophthalmopathy). The hormonal balance in certain patients before and after treatment with desiccated thyroid is also presented.

MATERIAL AND METHODS

The procedures for the use of the stasis tadpole as a bioassay test object, and the validity of the technique for simultaneous detection of thyroid and thyrotropic hormones in serum are discussed in detail elsewhere (13, 15, 16). The method involves induction of thyroid atrophy and metamorphic stasis in the tadpole by complete starvation. Intraperitoneal administration of either thyroid substance or TSH causes resumption of development; in the first instance, by supplying exogenously the thyroid hormone necessary for metamorphosis, and in the second, by stimulating the tadpole's thyroid to produce the endogenous thyroid hormone in which it is deficient. The metamorphic advance occurring after injection of different sera has been shown to represent additive effects of varying amounts of thyroid and thyrotropic hormone activity in the sample. The relative contribution of each component can be ascertained by precise measurement of the activity of either one of them. This has been done by previously establishing the thyroid response (cell-height increase) and consequent hindlimb extension following graded doses of purified TSH. The degree of thyroid stimulation obtained with the unknown sample is referred to the standard nomogram and the proportion of total hindlimb extension due to TSH alone in the blood sample can be calculated. Accordingly, the relative activities of both hormones in the blood become known, and thus the "thyroid-TSH" balance can be defined.

The blood donors in these experiments consisted of a series of presumably healthy individuals engaged in the laboratory, with no evident endocrine disease, and groups of patients in whom the clinical diagnosis

was either thyroid or pituitary gland derangement or possibly allied endocrinopathy. The majority of these individuals had been untreated for at least several months prior to blood withdrawal. In some, the difficulty had been one of long standing and was complicated by other disorders. A few patients were studied before and after treatment with desiccated thyroid. Basal metabolic rates were determined routinely, with blood cholesterol, protein-bound iodine, and radioiodine tracer uptake measured in selected cases. Sera were always frozen promptly, stored, and allowed to thaw only at the time of injection. Groups of sera were generally tested in parallel bioassay experiments (aliquots of the same sample yield essentially similar results despite varying periods of refrigeration). A control group of tadpoles was always tested along with the experimental groups, and at least 5 such control studies were made during the period of this investigation (two years).

RESULTS

Figure 1 depicts the progressive increase in thyroid cell height and the concomitant hindlimb extension in test animals following the administration of graded doses of a standard preparation of TSH (International Gland Preparation of desiccated Ox Anterior Pituitary). Application of the data obtained with normal sera (Table 1) to this standard nomogram reveals that TSH activity was detected in the majority, but not in all, of the cases tested. A total of 0.35 ml. of the raw serum given over seven injections, on alternate days, significantly raised the mean thyroid cell height (all cases combined) from 4.9 micra (control stasis value) to 5.6 micra. With these values it may be calculated from the standard nomogram that the mean TSH concentration detected, for all cases collectively, was equivalent to approximately 7.5 micrograms of standard per ml. of serum (0.0005 Junkmann-Schoeller unit; range, 0–0.001). It is apparent also (Fig. 1 and Table 1) that the mean hindlimb extension (4.7 mm.) in test animals was much greater than could be accounted for by the titers of TSH present (a hindlimb increase corresponding to a thyroid cell height of 5.6 micra would not exceed 1 mm.). Accordingly, analysis of these findings renders it likely that the metamorphosing activity of normal human serum reflects a thyroid-TSH balance in which the thyroid hormone component quantitatively exerts the dominant role.

Although the number of cases with known pituitary involvement is small (Table 2, Fig. 2) the results obtained with these sera are consistent. In the 3 cases of acromegaly the mean serum concentration of TSH was elevated approximately eightfold over the normal value. Hindlimb extension with these sera was also increased (in Case 2, 0.20 ml. of serum was sufficient to cause forelimb extrusion). Since the elevations in TSH titer

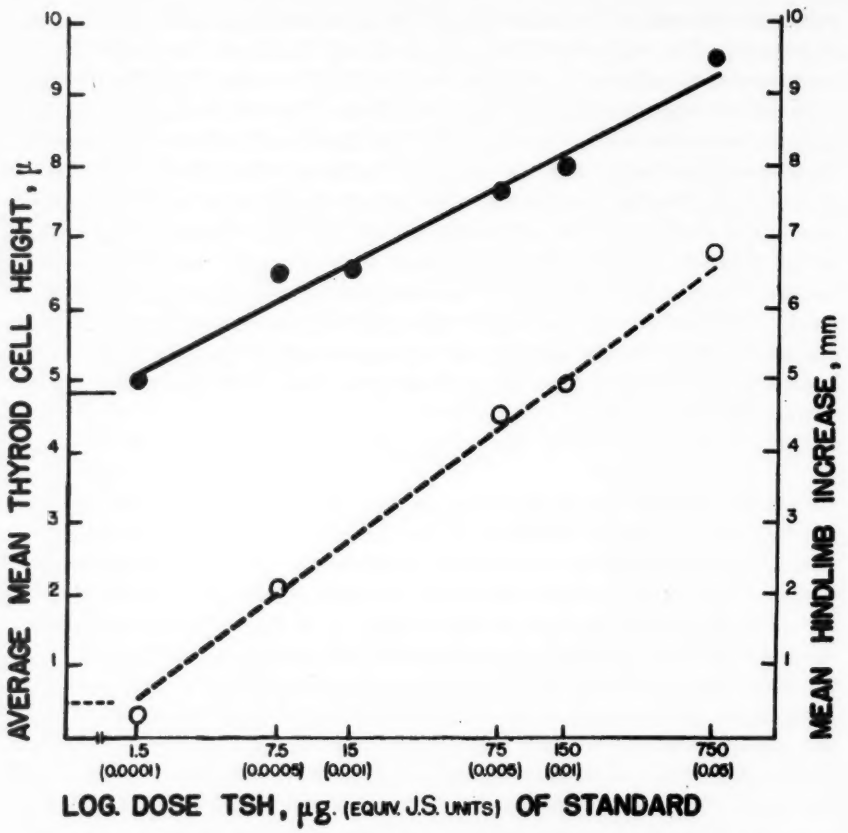


FIG. 1. Log dose-response curves indicating the increase in thyroid cell height (solid line—$Y = 1.54 \times 4.88$), and extension of hindlimb (broken line—$Y = 2.26 \times 0.12$) in the stasis tadpole with increasing doses of TSH (standard preparation of desiccated ox pituitary). Cell height at each point based on counts in 6–9 animals.

Horizontal bars on ordinate represent values in control animals. Equations for the lines were calculated by the method of least squares.

here were of sufficient magnitude to contribute appreciably to hindlimb development, it is likely that the increased hindlimb extension over that with normal serum represented augmented TSH rather than thyroid hormone activity (although some increase in the latter must have occurred in Case 2). It is evident, therefore, that in these acromegalics the thyroid-TSH equilibrium in the circulating blood was shifted from the normal in such fashion that thyroid hormone levels remained within or close to normal limits while TSH titers were markedly elevated. The blood thyroid-

TABLE 1. THYROID-TSH BALANCE IN NORMAL SERUM AS TESTED IN THE STASIS TADPOLE

Case No.	Donor-Sex-Age			Effect of serum in test tadpoles*		Estimated TSH conc.,† JSU/ml. serum
				Hindlimb increase (mm.)**	Thyroid cell height (μ)***	
1	Pooled	10 ♂	22–35	5.6±0.4	5.4±0.1	
2	Pooled	4 ♂	20–40	6.2±0.4	6.0±0.2	0.0010
3	Pooled	3 ♂3 ♀	30–40	5.2±0.3	5.8±0.1	0.0008
4	S.D. ♂		39	3.6±0.3	5.6±0.1	0.0006
5a	K.P. ♂		54	5.3±0.3	6.0±0.2	0.0010
5b	K.P.‡		54	5.1±0.4	5.1±0.1	
6	E.H. ♀		24	5.0±0.3	5.4±0.2	
7	E.D. ♀		37	3.0±0.2	5.9±0.1	0.0009
8a	A.G. ♀		24	4.5±0.4	5.7±0.1	0.0007
8b	A.G. ♀		24	4.3±0.3	5.6±0.1	0.0006
9a	M.K. ♀		24	4.1±0.5	5.7±0.1	0.0007
9b	M.K. ♀		24	4.9±0.1	5.4±0.1	
Combined Mean and S.E.				*4.7±0.3*	*5.6±0.1*	*(0.–0.001* JSU)
Range				*(3.0–6.2)*	*(5.1–6.0)*	
Saline control#				0.5±0.2	4.8±0.2	
Saline control				0.1±0.1	4.5±0.1	
Saline control				0.4±0.1	5.1±0.1	
Distilled water				0.3±0.1	5.2±0.1	
Hypx. rat serum§				0.1±0.1	5.1±0.1	
Combined mean and S.E.				*0.3±0.1*	*4.9±0.1*	
Range				*(0.1–0.5)*	*(4.5–5.2)*	

* From 10 to 15 tadpoles were used in each group; each animal received a total of 7 injections (0.05 cc. of serum or saline given on alternate days).

** Mean hindlimb increase (mm. ±standard error) over initial hindlimb length (similar in all test groups; 3.3 to 3.8 mm.).

*** Mean thyroid cell height in micra ±standard error. Based on counts made in 6 to 8 animals.

† Range of TSH concentration estimated from mean thyroid cell height by extrapolation on standard TSH curve (Fig. 1). Thyroid cell heights of 5.6μ or above are highly significant statistically; values below this have not been extrapolated, despite some measure of significance.

‡ Cases 5b; 8b; and 9b represent repeat experiments on the same individuals, with blood drawn approximately two months after the initial test.

Control stasis values ascertained at five separate times during the course of this investigation.

§ Hypophysectomized females (150–170 Gm.) three to four weeks after operation, with target organs atrophic.

TABLE 2. THYROID-TSH BALANCE IN SERA OF PATIENTS WITH PITUITARY DYSFUNCTION

Case-Donor Sex-Age		Clinical status	BMR, %	No. injections (total vol., cc.)	Effect of serum on test animal			Estimated TSH conc., JSU/ml. serum
					Mean hindlimb increase (mm.)	Body	Mean thyroid cell height (μ)	
1	A.Z. ♂-46	Acromegaly, untreated	− 6	5(0.25)	6.8±0.6		6.2±0.2	0.003
2	S.S. ♂-50	Acromegaly, untreated; bitemporal hemianopsia	− 8	4(0.20)	5.3±0.3	Forelimb* (70%)	6.4±0.1	0.004
3	M.P. ♀-57	Acromegaly, untreated	+23	7(0.35)	7.1±0.3		6.8±0.2	0.005
1	S.D. ♀-32	Simmonds' disease	−33	7(0.35)	0.2±0.1		5.3±0.1	
2a	E.S. ♀-47	Simmonds' disease	†	7(0.35)	0.1±0.1		5.3±0.1	
2b‡	E.S. ♀-47	Simmonds' disease	−23	7(0.35)	0.2±0.1		5.3±0.1	

* Seventy per cent of test tadpoles extruded forelimbs after 4 injections of serum. (Such bodily changes as forelimb extrusion or tail resorption indicate marked metamorphic stimulation.)

† Not taken.

‡ Repeat experiment—serum drawn four months later.

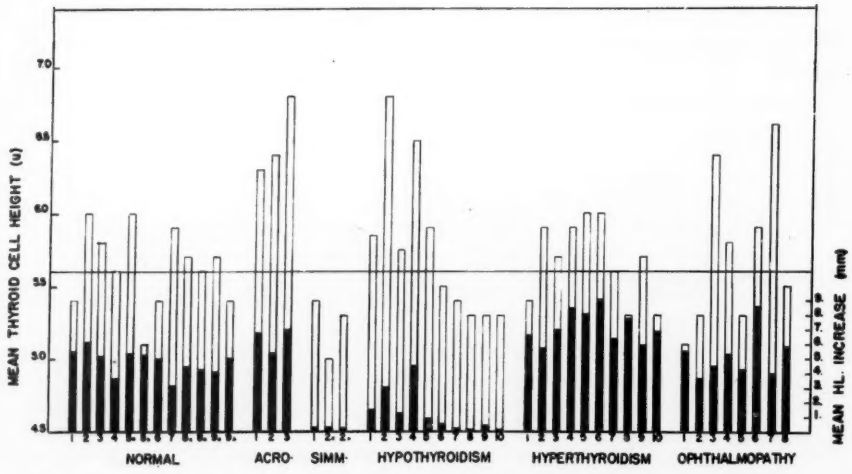


FIG. 2. A comparison of the metamorphic and thyroid-stimulating capacity of sera from normal and endocrine patients.

On the abscissa are represented the individual cases comprising the normal series (Table 1), and clinical groups of patients (Tables 2, 3, 4, 5) whose sera were tested in the stasis tadpole.

The height of the open bar indicates the mean thyroid cell height in the test animal group (TSH is considered detected with a cell height of 5.6μ—horizontal line—or above). The height of the darkened bar refers to the degree of hindlimb increase with the same sample of serum. That proportion of the total increase due to the thyroidal effect alone can be approximated by subtracting the hindlimb extension corresponding to the thyroid cell height as shown in the nomogram in Figure 1 (cell heights of 5.6μ–6.8μ are equivalent to hindlimb increases of 1 and 3 mm., respectively).

TSH balance in subjects with Simmonds' disease was also found to differ from the normal; however, values for both thyroid cell height and hindlimb development (Table 2, Fig. 2) were significantly decreased and approached stasis control values. Thus, the results in Simmonds' disease are in direct contrast to those found in acromegaly inasmuch as neither thyroid nor thyrotropic hormone could be detected in the blood.

TABLE 3. THYROID-TSH BALANCE IN SERA OF HYPOTHYROID INDIVIDUALS

Case-Donor-Sex-Age				Clinical status*	BMR, %	Effect of sera on test animal		Estimated TSH conc. JSU/ml. serum
						Mean increase (mm.)	Mean thyroid cell height (μ)	
1	M.C.	♀	50	Hypothyroidism	−31	1.5±0.1	5.8±0.1	0.0008
2	E.C.	♀	38	Myxedema (dermatitis)	−29	2.9±0.2	6.8±0.2	0.0050
3	L.H.	♀	68	Hypothyroidism	−23	1.3±0.3	5.7±0.2	0.0007
4	E.W.	♀	42	Hypothyroidism	−23	4.4±0.1	6.5±0.2	0.0040
5	F.N.	♀	54	Hypothyroidism (Riedel's struma)	−28	0.8±0.1†	5.9±0.1	0.0020
6	S.P.	♂	11	Cretinism‡		0.5±0.1	5.5±0.1	
7	E.M.	♀	41	Hypothyroidism	−27	0.2±0.1	5.4±0.1	
8	C.H.	♀	62	Hypothyroidism	−46	0.1±0.1	5.3±0.2	
9	D.T.	♀	29	Hypothyroidism	−22	0.3±0.1	5.3±0.1	
10	R.C.	♀	35	Hypothyroidism	−36	0.1±0.1	5.3±0.1	
Combined mean and S.E.						*1.2±0.1*	*5.8±0.1*	
Range						*(0.1–4.4)*	*(5.3–6.8)*	*(0.–0.095* JSU)

* Untreated for at least several months prior to time of blood withdrawal.

† Animals of this group received only 4 injections (total vol. 0.20 cc.) of serum; animals of all other groups received the usual 7 injections (0.35 cc.).

‡ BMR could not be determined; bone age, 2 years.

The effects of sera from hypothyroid patients (Table 3, Fig. 2) were consistently characterized by insignificant or relatively slight increases in hindlimb extension. The over-all decreased metamorphic response with these sera appeared to reflect primarily lowered thyroid hormone levels. In those instances in which hindlimb development was appreciable (Cases 2 and 4) the response could be accounted for largely by the increased titers of TSH found. Although the mean TSH level for all patients was not appreciably in excess of normal, TSH titers varied widely from patient to patient. Mean TSH concentrations were found to be either in the normal

range (Cases 1 and 3), excessively high (Cases 2, 4 and 5), or undetectable (Cases 6, 7, 8, 9 and 10). It is noteworthy that in this latter group the results were essentially similar to those found in Simmonds' disease, since neither thyroid nor thyrotropic hormone activity was demonstrable in the blood.

It is apparent from these data that in hypothyroidism the circulating blood is uniformly low in thyroid hormone content despite widely varying TSH titers.

The results obtained in the hyperthyroid patients (Table 4, Fig. 2) showed clearly that the metamorphosing activity of their sera was uniformly high. Hindlimb extension was markedly increased over that obtained with normal blood, and in most cases the metamorphic stimulus was so intense that advanced developmental changes (forelimb emergence)

TABLE 4. SERUM THYROID-TSH ACTIVITIES IN HYPERTHYROIDISM

Case-Donor Sex-Age	Clinical status*	BMR, %	No. injections (total vol., cc.)	Effect of serum on test animal			Estimated TSH conc., JSU/ml. serum
				Mean hindlimb increase (mm.)	Body	Mean thyroid cell height (μ)	
1 H.M. ♂44	Thyrotoxicosis	+42	5(0.25)	6.5±0.4	F.L.† (50%)	5.4±0.2	
2 W.L. ♂20	Thyrotoxicosis	+55	4(0.20)	5.8±0.3	F.L. (80%)	5.9±0.1	0.0020
3 J.Q. ♂30	Hyperthyroidism (diffuse type)	+40	7(0.35)	7.1±0.3	F.L. (20%)	5.7±0.1	0.0007
4 I.R. ♀30	Hyperthyroidism	+84	7(0.35)	8.5±0.3	F.L. (50%)	5.9±0.1	0.0009
5 J.O. ♂31	Thyrotoxicosis	+50	7(0.35)	8.3±0.2	F.L. (50%)	6.0±0.1	0.0010
6 O.A. ♂53	Hyperthyroidism (diffuse type)	+59	7(0.35)	9.0±0.4	F.L. (25%)	6.0±0.1	0.0010
7 L.H. ♀24	Thyrotoxicosis	+53	6(0.30)	6.4±0.4	F.L. (20%)	5.6±0.1	0.0006
8 E.R. ♀24	Thyrotoxicosis	+46	7(0.35)	7.8±0.5		5.3±0.2	
9 M.A. ♀50	Thyroid cancer	+30	7(0.35)	5.8±0.3		5.7±0.1	0.0007
10 J.C. ♀38	Hyperophthalmic Graves' dis.	+39	7(0.35)	6.8±0.7	F.L. (50%)	5.3±0.2	
Combined Mean and S.E.				*7.2±0.4*		*5.7±0.1*	
Range				*(5.8–9.0)*		*(5.3–6.0)*	*(0–0.002* JSU)

* Untreated for at least several months prior to time of blood withdrawal.
† Percentage of tadpoles extruding forelimbs.

TABLE 5. SERUM THYROID-TSH BALANCE IN OPHTHALMOPATHY

Case-Donor Sex-Age	Clinical status*	BMR, %	Effect of serum on test animal		Estimated TSH conc., JSU/ml. serum
			Mean hindlimb increase (mm.)	Mean thyroid cell (μ)	
1 C.P. ♀68	Exophthalmos (malignant)	+2	5.6±0.3	5.2±0.1	
2 E.A. ♀39	Exophthalmos	−2	3.7±0.5	5.4±0.1	
3 B.A. ♀47	Exophthalmos (ophthalmoplegia)	−3	4.5±0.4	6.4±0.2	0.0030
4 D.S. ♂30	Exophthalmos	+21	5.4±0.5	5.8±0.1	0.0008
5 E.G. ♀44	Exophthalmos	−15	4.3±0.3	5.4±0.1	
6 E.T. ♀69	Exophthalmos†	+39	8.6±0.3	5.9±0.1	0.0009
7 A.W. ♀46	Exophthalmos	−20	4.1±0.2	6.6±0.2	0.0050
8 M.S. ♀44	Exophthalmos (ophthalmoplegia)	−2	5.8±0.3	5.5±0.1	
Combined Mean and S.E.			*5.3±0.4*	*5.8±0.1*	
Range			*(3.7−8.6)*	*(5.2−6.6)*	*(0−0.005* JSU)

* All patients essentially untreated; in Case 8, however, the patient was receiving small inadequate doses of thyroid at the time of blood withdrawal.

† Thyrotoxic symptoms present, as well as ophthalmopathy.

occurred before the usual quota of injections was given. It was possible to predict the potential effects of these sera with as few as two injections (0.1 ml.) inasmuch as hindlimb increase was already evident at this time, contrasting sharply with the minimal or insignificant changes occurring in parallel experiments utilizing samples from the hypothyroid or Simmonds' disease series. These developmental effects were largely the result of thyroid hormone rather than TSH activity in the sera. Examination of the

thyroid cell heights indicates that in the majority of cases tested, TSH levels were detectable and existed in concentrations within the normal range. The general results, therefore, best describe for hyperthyroidism a thyroid-TSH balance in which an abnormally high thyroid hormone component co-exists with essentially normal titers of TSH.

Although in 2 patients with ophthalmopathy (Cases 3 and 7) the TSH activity of sera was found to be markedly elevated, the general results in this series (Table 5, Fig. 2) approximated those obtained with normal blood. Comparison of thyroid cell height and hindlimb extension data with those in the other series indicated that values for hindlimb increase repre-

TABLE 6. THYROID-TSH BALANCE IN SERUM OF PATIENTS BEFORE AND AFTER TREATMENT WITH DESICCATED THYROID

Case	Diagnosis	Treatment*	BMR, %	Effect of serum on test animal			Estimated TSH conc., μU/ml. serum
				Mean hindlimb increase (mm.)	Body	Mean thyroid cell height (μ)	
1	Exophthalmos (malig.)	Before:	+ 2	5.6±0.3		5.2±0.1	
		After: 4 gr. daily; 6 months:	− 2	6.8±0.7†	Tail resorption (100%)	6.5±0.2	0.0050
3	Exophthalmos	Before:	− 3	4.5±0.4		6.4±0.2	0.0030
		After: 1-3 gr. daily; 6 weeks:	+21	6.0±0.4		6.7±0.1	0.0040
1	Hypothyroidism	Before:	−31	1.5±0.1		5.8±0.1	0.0008
		After: 1 gr. daily; 3 months:	+ 6	1.8±0.1		6.1±0.2	0.0010
		4 months:	—	2.8±0.2		6.3±0.1	0.0020
2	Hypothyroidism	Before:	−29	2.9±0.2		6.8±0.2	0.0050
		After: 1-2½ gr. daily; 6 months:	+11	7.0±0.3‡	Forelimb (90%)	6.2±0.1	0.0030
3	Hypothyroidism	Before:	−23	1.3±0.3		5.7±0.	0.0007
		After: 1 gr. daily; 6 weeks:	−17	2.3±0.1		5.5±0.1	0.0005
		15 weeks:	− 7	2.6±0.2		5.6±0.1	0.0006
10	Hypothyroidism	Before:	−36	0.1±0.1		5.3±0.1	
		After: 1 gr. daily; 2 months:	− 4	1.7±0.2		5.3±0.1	

* All patients clinically improved after treatment.

†,‡ The effects after 4 and 5 injections of serum, respectively. In all other instances, 7 injections totalling 0.35 cc. were given.

sented, as in the normal series, preponderantly high thyroid hormone rather than TSH activity. The thyroid-TSH relationship, however, appears to have been altered in Cases 3 and 7. The TSH values here were sufficiently high to expect greater hindlimb development than was present

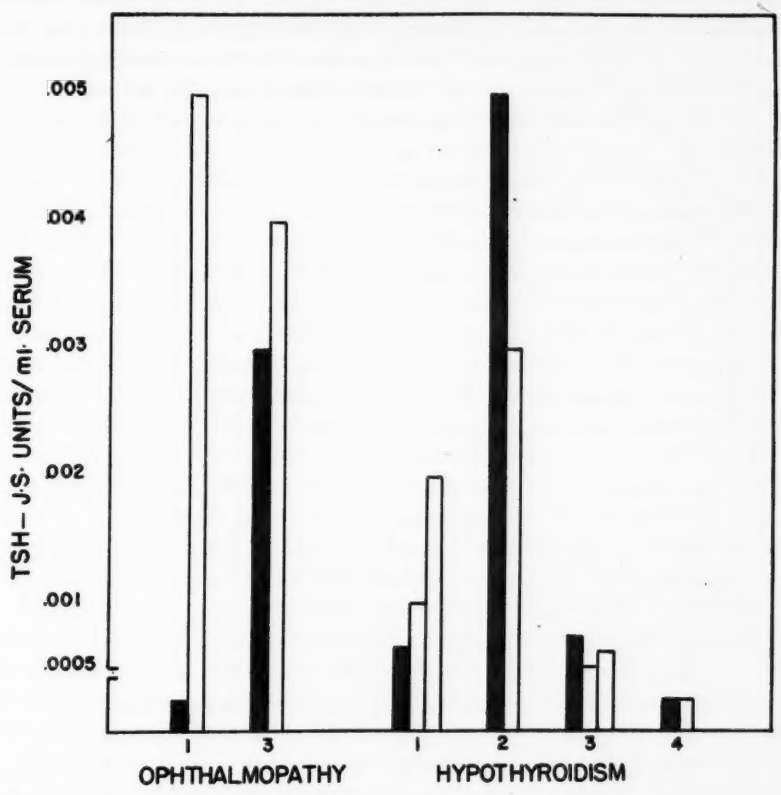


FIG. 3. Blood concentrations of TSH in patients before and after treatment with desiccated thyroid.

The dark and light bars refer, respectively, to the TSH concentration (JSU/ml. serum) in the blood of the patient before and after treatment indicated in Table 6.

and it is likely that the heightened TSH titers were accompanied by reduced thyroid hormone levels. These changes could not be correlated with severity of symptoms; marked exophthalmos with ophthalmoplegia existed with essentially normal thyroid-TSH balance in the blood in Case 8.

The responses obtained with sera from patients before and after treatment with desiccated thyroid gland were summarized and contrasted (Table 6, Fig. 3). It is seen that in both cases of ophthalmopathy clinical

improvement occurred in the presence of abnormally high titers of TSH in the blood. In Case 1, thyroid administration resulted in marked elevation of the TSH concentration from a previously undetectable level, whereas in Case 3 the initially high TSH level was maintained. A similar situation was found in 2 of the 4 hypothyroid subjects. In Cases 1 and 2 of this series clinical improvement, following thyroid ingestion, co-existed with blood levels of TSH definitely above normal. In the remaining subjects (Cases 3 and 10), however, clinical improvement was effected without significant change in the blood thyrotropic activity, which remained relatively low.

Among patients in the same clinical category, differences in the peripheral response to desiccated thyroid gland are also revealed (Table 6). In Case 1 (exophthalmos), considerable amounts of exogenous thyroid hormone must have accumulated in the blood as indicated by the fact that injection of only 0.25 ml. of serum was sufficient to transform all test animals to practically the frog stage; nevertheless, the basal metabolic rate of the patient was unchanged. In Case 3 (exophthalmos) the basal metabolic rate was significantly elevated after treatment, but blood thyroid hormone levels were not appreciably augmented. In both instances, TSH levels were elevated equally. Two patients (Cases 1 and 2) of the hypothyroid series showed approximately equal and appreciable increases in the BMR with thyroid treatment; however, the blood thyroid hormone level in the former remained below normal, whereas in the latter it became excessively high (as indicated by forelimb extrusion in the test animals). The blood TSH level was increased in both instances.

Although the number of cases in this series is small, the results indicate that ophthalmopathy and hypothyroidism can exist in individuals with either high or low TSH levels in blood; moreover, amelioration of clinical symptoms with desiccated thyroid gland can occur without restoration of a normal thyroid-thyrotropic hormone balance in the circulating blood.

DISCUSSION

The supposition that the thyroid and pituitary are reciprocally related has led many investigators to attempt detection and definition of TSH levels in the body fluids of thyroid patients. The results, however, have been largely negative or inconclusive (1, 17, 18) and little or no indication of the physiologic or pathologic role of the hypophysis in thyroid disease has been provided by this approach. It is now apparent that the numerous discrepant reports regarding the presence or absence of TSH in blood and urine stemmed from methods too crude to detect the low titers of hormone present in the body fluids. The present study demonstrates that endogenous TSH can be detected in untreated serum of normal individuals de-

spite its low concentration (range, 0–0.001 JSU/ml. serum). Although DeRobertis (10) finds it necessary to remove the thyroid hormone fraction before TSH activity becomes manifest, it is noteworthy that the values reported for TSH in normal blood (3 cases) fall, after proper correction,[1] within this range. Present estimations of the blood TSH, nevertheless, must be considered tentative; full definition of the range of concentration awaits numerous tests in different age and sex groups.

Although the number of patients with diagnosed pituitary dysfunction was small, the consistency of the results in this study lend credence to the frequently suggested probability that hyperpituitarism and hypopituitarism are associated, respectively, with increased and decreased production of TSH. In both acromegaly and Simmonds' disease the deviation from normal in the blood thyroid-TSH balance was appreciable and in opposite directions. The findings in Simmonds' disease are readily understandable; as a result of pituitary destruction, there is no TSH production and thyroid hormone secretion consequently fails. The presence, in acromegaly, of high blood titers of TSH with apparently absent "target" response of the thyroid is unexpected and difficult to explain. Previous reports on TSH levels in body fluids of acromegalics have been contradictory (11, 19, 20, 21), as have been those on blood iodine (22, 23). The demonstration of normal thyroid hormone concentrations, in the present study, is in accord with results of recent investigations (24, 25, 26) which indicate that the radioiodine uptake of the thyroid in acromegaly is within normal limits. The significance of the elevated TSH titers is not clear. The clinical manifestations of acromegaly are best explained as resulting from excessive growth hormone secretion from the pituitary. It is probable that the high TSH levels represent augmented release of this hormone from the deranged pituitary. Whether or not both pituitary principles can exist simultaneously at high levels in the blood is not known. Acromegaly is characterized by periods of presumably increased hormonal activity alternating with inactive periods. The patients of this study, at the time of examination, were in the inactive phase; all had a normal basal metabolic rate, and none had goiter. A clearer understanding of the hormonic balance in acromegaly awaits bioassays of the thyrotropic and growth principles in both phases of the disease.

There are many experimental observations which support the concept that the interrelated activities of the thyroid and the anterior hypophysis are delicately balanced by the blood levels of their hormones. It is amply

[1] An unfortunate error exists in this report as regards extrapolation of the cytologic coefficients of normal bloods from the standard curve. Extrapolated values are less than those indicated; moreover, they represent the TSH activity in 2 ml. rather than in 1 ml. of whole blood.

clear that in the present study the thyroid-TSH levels in the blood of hyperthyroid or hypothyroid patients did not conform to those expected if reciprocal regulatory control were operative at the time of blood withdrawal. Despite the consistently low levels of blood thyroid hormone, only 2 of the 10 hypothyroid individuals tested showed TSH titers which could be regarded as excessively high. In hyperthyroid subjects, in whom thyroid hormone levels were characteristically high, the TSH concentrations were generally in the normal range. It is possible that in those cases of hypothyroidism in which TSH was not detected there may have been a primary disturbance of the thyrotropic mechanism in the hypophysis; in this regard, it may be significant that the thyroid-TSH balance in these individuals was indistinguishable from that in patients with Simmonds' disease. A study of the hormonic balance in the circulating blood of both types of patients after exogenous TSH administration is, therefore, indicated.

There are many reports in the literature to the effect that hyperthyroid individuals have little or no TSH in the blood or urine. Since the methods employed have generally been too insensitive to demonstrate TSH in normal body fluids, it is likely that TSH titers in the blood of hyperthyroid patients may frequently have gone undetected. The presumably normal amount of circulating thyrotropin in thyrotoxicosis, as demonstrated in this study, indicates that the hyperplasia and excessive activity of the thyroid gland in such patients cannot be due to abnormal TSH concentrations in the blood stream. It has been postulated from *in vivo* and *in vitro* studies (27, 28, 29), and subsequently questioned (30), that in toxic goiter the pituitary produces excess TSH but that this is quickly inactivated by the hyperplastic thyroid tissue. Whether the sensitivity of the target gland to normal amounts of circulating TSH is appreciably changed in hyperthyroidism remains to be tested.

Recently Dobyns (31) has critically evaluated the anatomic, pathologic, and clinical data bearing on the problem of exophthalmos. There is much to implicate the pituitary in the pathogenesis of this disease. The connective tissue reactions in the retrobulbar tissue of the patient are similar to those occurring in animals rendered exophthalmic with pituitary preparations, presumably TSH. Increased prominence of the eyes follows thyroidectomy or administration of thiouracil, and regression occurs with desiccated thyroid—procedures which in themselves are considered to induce high and low titers of TSH, respectively. Roentgen irradiation or electrocauterization of the pituitary improves the ophthalmopathy. The present study demonstrates, however, that the syndrome can exist without apparent relation to the levels of TSH in the blood inasmuch as ophthalmopathy was present with thyrotropic hormone either absent, normal,

or excessively high, in the blood. It appears significant, moreover, that in 2 patients the ocular signs subsided after thyroid administration, even though the blood TSH values remained high, or subsequently achieved high levels. It should also be mentioned that no ocular changes were noted in other cases of this study in which TSH levels were high (acromegaly, hypothyroidism). Our findings thus support the earlier contention by Purves and Griesbach (30) that a causal role of TSH in the production of malignant exophthalmos is unlikely.

It seems particularly interesting that in 4 of the 6 patients treated with thyroid substance the blood TSH either rose to high levels from previous low ones or maintained its initially high concentrations. It is evident that with doses of thyroid sufficient to improve clinical status the effect on the thyrotropic mechanism of the pituitary need not be depressant and may be excitatory. In an earlier study, Starr, Rawson and their colleagues (32) noted that the pituitary failed to release TSH in profound myxedema but did so when small amounts of exogenous thyroid were supplied. Clinical improvement with amounts of thyroid which are not capable of suppressing excessive TSH values may perhaps be analogous to the clinical improvement in cases of menopausal syndrome treated with estrogens in doses too small to decrease high FSH excretion. Similarly, the augmentation of TSH secretion by thyroid medication may be comparable to the rise of gonadotropin titers occasionally observed in women treated with small doses of estrogen (33). The fact that TSH secretion can occur at high levels in patients clinically normalized by thyroid medication makes it worthwhile to determine whether the augmented TSH secretion can be long maintained and whether other functions of the anterior hypophysis are eventually impaired.

SUMMARY AND CONCLUSIONS

The status of anterior pituitary-thyroid gland interplay in normal subjects and endocrine patients has been investigated by determining the levels of thyroid and thyrotropic hormone activities in untreated serum and correlating these with the clinical state.

The metamorphosing capacity of normal sera in test tadpoles was found to reflect a thyroid-TSH equilibrium in which TSH activity was of a low order of magnitude (range, 0–0.001 JSU/ml. serum), with thyroid hormone activity relatively high. In acromegaly (3 cases), blood titers of TSH were significantly increased (approximately eightfold), whereas thyroid hormone levels remained near normal limits. Neither thyroid nor TSH was demonstrable in the blood of patients with Simmonds' disease (3 cases). In hypothyroidism (10 cases) thyroid hormone activity in the blood was uniformly low but TSH titers varied widely (0–0.005 JSU/ml. serum). Con-

versely, in hyperthyroidism (10 cases) excessively high thyroid hormone levels in the blood usually co-existed with a normal TSH content. The thyroid-TSH balance in ophthalmopathy was essentially normal, although elevated TSH levels did occur (2 cases). Clinical improvement in exophthalmic and hypothyroid individuals could be effected (with desiccated thyroid) in the presence of abnormally high TSH concentrations in the serum.

The results indicate that the thyroid-TSH balance in the circulating blood is frequently altered in patients with thyroid or pituitary malfunction, and that amelioration of clinical symptoms can occur without restoration of the normal hormonic balance.

REFERENCES

1. ADAMS, E. A.: Variations in the potency of thyrotrophic hormone of the pituitary in animals, *Quart. Rev. Biol.* **21:** 1–32, 1946.
2. SALTER, W. T.: The Endocrine Function of Iodine, Cambridge, Harvard University Press, 1940.
3. MEANS, J. H.: The Thyroid and Its Diseases, Philadelphia, J. B. Lippincott Co., 1948.
4. RAPPORT, R. L., and CURTIS, G. M.: The clinical significance of the blood iodine: a review, *J. Clin. Endocrinol.* **10:** 735–790, 1950.
5. STARR, P.; PETIT, D. W.; CHANEY, A. L.; ROLLMAN, H.; AIKEN, J. B.; JAMIESON, B., and KLING, I.: Clinical experience with the blood protein-bound iodine as a routine procedure, *J. Clin. Endocrinol.* **10:** 1237–1250, 1950.
6. WERNER, S. C.; HAMILTON, H. B.; LEIFER, E., and GOODWIN, L. D.: An appraisal of the radioiodine tracer technic as a clinical procedure in the diagnosis of thyroid disorders, *J. Clin. Endocrinol.* **10:** 1054–1076, 1950.
7. CURTIS, G. M., and SWENSON, R. E.: The significance of the protein-bound iodine in patients with hyperthyroidism, *Ann. Surg.* **128:** 443–455, 1948.
8. STANLEY, M. M., and ASTWOOD, E. B.: The response of the thyroid gland in normal human subjects to the administration of thyrotropin, as shown by studies with I^{131}, *Endocrinology* **44:** 49–60, 1949.
9. QUERIDO, A., and STANBURY, J. B.: The response of the thyroid gland to thyrotropic hormone as an aid in the differential diagnosis of primary and secondary hypothyroidism, *J. Clin. Endocrinol.* **10:** 1192–1201, 1950.
10. DE ROBERTIS, E.: Assay of thyrotropic hormone in human blood, *J. Clin. Endocrinol.* **8:** 956–966, 1948.
11. DEL CASTILLO, E. B., and MAGDALENA, A.: Hypophyse et thyroïde. Pouvoir excito-thyroïdien du sérum sanguin, *Compt. rend. Soc. de biol.* **108:** 917–918, 1931.
12. D'ANGELO, S. A.; GORDON, A. S.; PASCHKIS, K. E., and CANTAROW, A.: Thyrotropic and thyroid hormone assay of normal and pathologic human sera in the stasis tadpole, (abstract) *J. Clin. Endocrinol.* **9:** 653, 1949.
13. D'ANGELO, S. A., and GORDON, A. S.: The simultaneous detection of thyroid and thyrotrophic hormones in vertebrate sera, *Endocrinology* **46:** 39–54, 1950.
14. D'ANGELO, S. A., and GORDON, A. S.: Thyroid-thyrotropic hormone equilibria in body fluids, as tested in the starved tadpole, *Tr. Am. Goiter A.* pp. 140–144, 1949.
15. D'ANGELO, S. A.; GORDON, A. S., and CHARIPPER, H. A.: The role of the thyroid

and pituitary glands in the anomalous effect of inanition on amphibian metamorphosis, *J. Exper. Zoöl.* **87:** 259–277, 1941.

16. D'Angelo, S. A.; Gordon, A. S., and Charipper, H. A.: Thyrotropic hormone assay in the tadpole, *Endocrinology* **31:** 217–225, 1942.
17. Collip, J. B.: Corticotropic (adrenotropic), thyrotropic and parathyrotropic factors, *in* Glandular Physiology and Therapy, ed. 2, Chicago, Ill., American Medical Association, 1942, pp. 33–50.
18. Albert, A.: The biochemistry of the thyrotropic hormone, *Ann. New York Acad. Sci.* **50:** 466–490, 1949.
19. Fellinger, K.: Klinische und experimentelle Untersuchungen über das Verhalten und die Bedeutung des thyreotropen Hormons in Blute, *Wien. Arch. f. inn. Med.* **29:** 375–406, 1936.
20. Nitzescu, I. I., and Timus, D.: Die Ausscheidung des thyreotropen Hormons aus dem Hypophysenvorderlappen durch den Harn bei Akromegalie, *Spitalul* **58:** 5, 1938.
21. Jones, M. S.: A study of thyrotropic hormone in clinical states, *Endocrinology* **24:** 665–671, 1939.
22. Perkin, H. J., and Lahey, F. H.: The level of iodine in the blood, *Arch. Int. Med.* **65:** 887–895, 1940.
23. Curtis, G. M., and Fertman, M. B.: Blood iodine studies, *Arch. Surg.* **54:** 541–554, 1947.
24. McCullagh, E. P.; Gold, A., and McKendry, J. B. R.: Radioactive iodine uptake in the hypermetabolism of acromegaly, *J. Clin. Endocrinol.* **10:** 687–691, 1950.
25. Werner, S. C.; Quimby, E. H., and Schmidt, C.: The use of tracer doses of radioactive iodine, I^{131}, in the study of normal and disordered thyroid function in man, *J. Clin. Endocrinol.* **9:** 342–354, 1949.
26. Keating, F. R., Jr.; Haines, S. F.; Power, M. H., and Williams, M. M. D.: The radioiodine-accumulating function of the human thyroid gland as a diagnostic test in clinical medicine, *J. Clin. Endocrinol.* **10:** 1425–1464, 1950.
27. Seidlin, S. M.: The metabolism of the thyrotrophic and gonadotrophic hormones, *Endocrinology* **26:** 696–702, 1940.
28. Rawson, R. W.; Graham, R. M., and Riddell, C. B.: Physiological reactions of the thyroid stimulating hormone of the pituitary. II. The effect of normal and pathological human thyroid tissues on the activity of the thyroid stimulating hormone, *Ann. Int. Med.* **19:** 405–414, 1943.
29. Thompson, W. O., and Thompson, P. K.: The role of the pituitary in toxic goiter, (abstract) *Endocrinology* **35:** 206, 1944.
30. Purves, H. P., and Griesbach, W. E.: Thyrotropic hormone in thyrotoxicosis, malignant exophthalmos, and myxoedema, *Brit. J. Exper. Path.* **30:** 23–30, 1949.
31. Dobyns, B. M.: Present concepts of the pathologic physiology of exophthalmos, *J. Clin. Endocrinol.* **10:** 1202–1230, 1950.
32. Starr, P.; Rawson, R. W.; Smalley, R. E.; Doty, E., and Patton, H.: The microhistometric method applied to thyrotropic hormone assay, *West. J. Surg.* **47:** 65–75, 1939.
33. Heller, C. G.; Chandler, R. E., and Myers, G. B.: Effect of small and large doses of diethylstilbestrol upon menopausal symptoms, vaginal smear and urinary gonadotrophins in 23 oöphorectomized women, *J. Clin. Endocrinol.* **4:** 109–116, 1944.

PROTEIN-BOUND IODINE IN SERUM OF RATS RECEIVING IODIDE OR IODINE WITH OR WITHOUT GOITROGEN*†

T. S. DANOWSKI, M.D., SUZANNE J. HUFF, M.A., PATRICIA M. WIRTH, M.S., G. C. HILL, B.S., MARY H. GREEN, M.D. AND G. H. FETTERMAN, M.D.

From the Departments of Research Medicine and Pathology, the Children's Hospital, and the Renziehausen Foundation, University of Pittsburgh School of Medicine, Pittsburgh, Pa.

DVOSKIN'S studies with elemental iodine (1, 2, 3) clearly established that injections of this form of the halogen were as effective as thyroxine or desiccated thyroid in cancelling the inhibition of growth which followed thyroidectomy or goitrogen therapy in immature rats. Subsequently Barker showed that administration of elemental iodine by the subcutaneous route produced marked elevations of the protein-bound or precipitable iodine in the plasma of rats and in the tissues at the sites of injection (4, 5). In the experiments reported by Dvoskin, parenteral or oral inorganic iodide proved to be essentially inert in restoring or maintaining normal growth rates in hypothyroid or athyroid rats. These findings are of particular interest, since Chaikoff had observed (6) that in rats fed inorganic iodide the protein-bound iodine of serum also rose, though these rises were of lesser magnitudes than those described in Barker's work. Our own studies in this field (7–10), conducted in humans, indicated that oral feeding of potassium iodide in massive or even in ordinary therapeutic amounts raises the nonthyroxine fraction of the protein-bound iodine without producing hypermetabolism.

In view of the above facts we have undertaken this series of studies in rats to see whether or not goitrogenic agents influence the protein-bound iodine changes observed after inorganic iodide or elemental iodine therapy.

MATERIALS AND METHODS; EXPERIMENTAL PROCEDURE

Potassium iodide, by mouth or by injection, or elemental iodine by in-

Received for publication July 19, 1951.

* Read at the Annual Meeting of the American Goiter Association, Columbus, Ohio, May 24, 1951.

This article will be included in the bound volume of the "Transactions of the American Goiter Association" published by Charles C Thomas, Publisher, which will be available for sale early in 1952.

† This investigation was supported in part by a research grant from the Division of Research Grants, of the National Institutes of Health, Public Health Service.

TABLE 1. SERUM PROTEIN-BOUND IODINE IN FEMALE WISTAR RATS TREATED WITH GOITROGEN OR POTASSIUM IODIDE, ALONE OR IN COMBINATION

Experiment	No. of rats		Therapy	Mean body weight (grams)		Protein-bound iodine of serum, at finish	Comment*
	Start	Finish		Start	Finish	(γ% mean ±S.D.)	
I	a) 10	9	None	111.7	152.6	2.2±0.6	Rise in PBI induced by KI was partially cancelled by goitrogen.
23 days;	b) 10	9	Propylthiouracil 0.2%	115.1	109.4	1.1±0.9	
KI subcut.	c) 10	5	KI −2 ml. of 0.5 Gm.%/day	113.5	142.7	8.1±3.1	
	d) 10	6	KI +propylthiouracil, as in b) and c)	114.7	137.2	3.7±2.8	
II	a) 7	4	None	137.0	188.7	2.6±0.7	Rise in PBI following inorganic iodide not affected by propylthiouracil.
74 days;	b) 10	9	Propylthiouracil 0.2%	138.1	127.6	0.3±0.4	
KI subcut.	c) 10	6	KI−2 ml. of 0.5 Gm.%/day	133.9	180.5	5.6±2.2	
	d) 10	8	KI +propylthiouracil, as in b) and c)	132.7	142.0	4.1±0.4	
III	a) 16	15	None	108.8	136.3	6.4±1.9	No statistically significant differences present.
17 days;	b) 16	14	Propylthiouracil 0.5%	99.9	108.3	8.6±3.0	
KI subcut.	c) 16	15	KI −2 ml. of 0.9 Gm.%/day	100.6	129.1	8.6±2.7	
	d) 16	14	KI +propylthiouracil, as in b) and c)	102.3	96.3	6.0±4.4	
IV†	a) 9	5	None	—	125.8	6.9±0.9	Propylthiouracil without effect on KI-induced rise in PBI.
23 days;	b) 15	7	Propylthiouracil 0.2%	—	74.8	4.2±1.5	
KI subcut.	c) 15	10	KI −2 ml. of 0.5 Gm.%/day	—	107.5	12.9±2.1	
	d) 15	6	KI +propylthiouracil, as in b) and c)	—	77.3	13.9±4.3	
V†	a) 9	5	None	—	125.8	6.9±0.9	Oral KI raised PBI; effect partially cancelled by goitrogen.
23 days;	b) 15	7	Propylthiouracil 0.2%	—	74.8	4.2±1.5	
KI by mouth	c) 15	12	KI −10 mg./15 Gm. of food	—	91.3	14.8±6.4	
	d) 15	9	KI +propylthiouracil, as in b) and c)	—	83.2	7.0±2.3	
VI	a) 20	10	Propylthicuracil 0.2%	98.7	112.5	1.3±0.7	Rise in PBI induced by KI ingestion totally cancelled by propylthiouracil
29 days;	b) 20	14	KI −10 mg./15 Gm. of food	102.0	149.5	14.8±4.3	
KI by mouth	c) 20	9	KI +propylthiouracil, as in a) and b)	101.5	120.8	1.6±1.9	

* Following application of "t" test and determination of "p"; value of less than 0.05 for "p" taken to be statistically significant.

† Control and goitrogen-treated animals common to Experiments IV and V.

jection has been administered to growing female rats of the Wistar strain, approximately four weeks old, during periods of time varying in length from seven to seventy-four days. In the inorganic iodide studies the control series consisted of untreated animals; in the elemental iodine experiments the controls received propylene glycol in the volumes used to dissolve the administered halogen. The effects of propylthiouracil ingestion upon the serum protein-bound iodine have been determined in both sets of studies. The details of the individual experiments have been incorporated into the tables. Barker's method has been modified (9–11) and employed

in measurements of the serum protein-bound or serum precipitable iodine (PBI or SPI).

RESULTS

A. *PBI of rats receiving inorganic iodide by mouth or subcutaneously without or with concomitant propylthiouracil ingestion*

The protein-bound iodine increased significantly above control values in 5 of the 6 experiments in which potassium iodide was administered by injection or in food (Table 1). The concomitant administration of a goitrogen, 0.2 per cent of propylthiouracil in food, interfered with or prevented the inorganic iodide effect upon protein-bound iodine in both of the oral studies (Experiments V and VI) and in 1 of the parenteral iodide studies (Experiment I). In 2 others propylthiouracil did not cancel the iodide-induced rise in circulating protein-bound iodine (Experiments II and IV). Finally, it should be pointed out that the results in Experiment III are unlike any of the others in that neither goitrogen nor iodide, alone or together, discernibly influenced the protein-bound iodine values. The experimental procedure differed from the rest, however, in that larger amounts of goitrogen and of iodide were given for a shorter period of time. In keeping with the findings of others, the goitrogen interfered with body growth.

TABLE 2. SERUM PROTEIN-BOUND IODINE IN FEMALE WISTAR RATS TREATED WITH GOITROGEN OR ELEMENTAL IODINE, ALONE OR IN COMBINATION

Experimental	No. of rats		Therapy	Mean body weight (grams)		Protein-bound iodine of serum, at finish (γ% mean ±S.D.)	Comment*
	Start	Finish		Start	Finish		
I	a) 7	5	Control (propylene glycol)	137.0	173.0	10.8± 3.5	Goitrogen without effect on rise in PBI induced by elemental iodine.
30 days;	b) 8	6	Propylthiouracil 0.2%	151.8	148.5	2.1± 3.0	
I_2 subcut.†	c) 8	7	I_2, 1.0 cc. of 1.0 Gm.% in propylene glycol	155.0	176.8	820.0± 87.7	
	d) 8	5	Propylthiouracil+I_2, as in b) and c)	151.0	154.1	883.6±399.0	
II	a) 16	13	Control (propylene glycol)	107.1	146.8	4.0± 1.2	Goitrogen augmented the rise in PBI induced by elemental iodine.
14 days;	b) 16	16	Propylthiouracil 0.5%	98.9	88.7	2.7± 2.3	
I_2 subcut., then 7 days rest†	c) 16	14	I_2, 1.0 cc. of 0.3 Gm.% in propylene glycol	104.5	152.0	51.5± 9.3	
	d) 16	11	I_2+propylthiouracil, as in b) and c)	102.3	100.9	111.0± 36.6	
III	a) 20	20	Control (propylene glycol)	113.2	137.1	15.7± 2.3	Goitrogen augmented the rise in PBI induced by elemental iodine.
7 days;	b) 20	16	Propylthiouracil 0.5%	113.8	109.4	5.7± 2.5	
I_2 subcut., then 7 days rest†	c) 20	16	I_2, 1.0 cc. of 0.3 Gm.% in propylene glycol	100.1	129.3	40.2± 8.2	
	d) 20	17	I_2+propylthiouracil, as in b) and c)	118.1	105.4	94.2± 23.5	

* Following application of "t" test and determination of "p"; values of less than 0.05 for "p" taken to represent statistically significant differences.

† Goitrogen administered throughout the experiment to groups b) and d).

B. *Changes in PBI of rats injected with elemental iodine*

In each of the 3 experiments in Table 2 injections of elemental iodine (1.0 ml. of either 1.0 or 0.3 gram per cent iodine in propylene glycol) resulted in marked increases in protein-bound iodine. In Experiments II and III the simultaneous ingestion of goitrogen incorporated into the diet at a level of 0.5 per cent further enhanced the rise of protein-bound iodine noted to follow the subcutaneous administration of elemental iodine. In all 3 experiments the highest gains in body weight were recorded in the elemental iodine group, whereas therapy with goitrogen alone either prevented growth or actually resulted in a loss of body weight. There is no evidence that the combination of elemental iodine and goitrogen cancelled the growth-inhibiting effects of the latter. This may well be related in Experiments II and III to the fact that goitrogen ingestion continued after the injections of iodine had been stopped. Experiment I is not comparable to those which follow, since its duration and the dosage of iodine and of propylthiouracil were not the same.

DISCUSSION

The described findings establish that the alterations which occur during potassium iodide treatment can be minimized or cancelled by the simultaneous administration of a goitrogen, whereas those induced by elemental iodine are augmented. Our studies do not identify, however, the site or sites at which the protein-bound iodine is produced by these agents. If these be in tissues other than the thyroid, as indicated by Barker's study (4), then evidence has been presented of extrathyroidal actions of goitrogens.

Preliminary observations based upon butanol extraction of serum and subsequent separation with sodium hydroxide and sodium carbonate (12–14) suggest that only a small portion of the rise in serum protein-bound iodine following administration of elemental iodine is attributable to changes in the thyroxine fraction (15). This increment is nonetheless of sufficient magnitude to provide a biochemical correlate of the biologic and histologic work of Dvoskin in which the suppressive effects of thyroidectomy or goitrogen therapy on growth were successfully overcome by injection of elemental iodine.

CONCLUSIONS

1) Inorganic iodide administered orally or parenterally raises the serum protein-bound iodine.

2) There is evidence that concomitant goitrogen therapy can at times minimize or cancel such changes.

3) The rise in protein-bound iodine which follows the subcutaneous administration of elemental iodine can at times be augmented by the simultaneous administration of a goitrogen.

Addendum

Since submission of this paper, we have conducted 3 additional experiments with elemental iodine and goitrogen. In 2 of the 3 studies, combined therapy with elemental iodine and propylthiouracil resulted in significantly greater increases in PBI: 330.8 versus 215.9 γ per cent, and 137.4 versus 76.0 γ per cent, respectively. This augmentative effect was not observed in the third experiment.

REFERENCES

1. Dvoskin, S.: The thyroxine-like action of elemental iodine in the rat and chick, *Endocrinology* **40:** 334–352, 1947.
2. Dvoskin, S.: Further observations on the thyroxine-like action of elemental iodine in the rat, *Endocrinology* **41:** 331–333, 1947.
3. Dvoskin, S.: The thyroxine-like action of elemental iodine, *Tr. Am. Goiter A.*, pp. 160–166, 1949.
4. Barker, S. B., and Lipner, H. J.: The effect of iodine injections on energy metabolism and plasma protein-bound iodine of rats, (abstract) *J. Clin. Endocrinol.* **8:** 596, 1948.
5. Barker, S. B., and Lipner, H. J.: *In vivo* iodination of tissue protein following injection of elemental iodine, *Science* **108:** 539, 1948.
6. Taurog, A., and Chaikoff, I. L.: The relation of the thyroxine content of the thyroid gland and of the level of protein-bound iodine of plasma to iodine intake, *J. Biol. Chem.* **165:** 217–222, 1946.
7. Danowski, T. S., and Greenman, J. H.: Alterations in serum iodine fractions during the administration of potassium iodide, *Tr. Am. Goiter A.* pp. 154–159, 1949.
8. Danowski, T. S., and Greenman, J. H.: Rise in serum precipitable or protein-bound iodine following massive doses of inorganic iodide, *Fed. Proc.* **8:** 31–32, 1949.
9. Danowski, T. S.; Mateer, F. M.; Weigand, F. A.; Peters, J. H., and Greenman, J. H.: Serum iodine fractions in subjects receiving potassium iodide in small dosage, *J. Clin. Endocrinol.* **10:** 532–539, 1950.
10. Danowski, T. S.; Johnston, S. Y., and Greenman, J. H.: Alterations in serum iodine fractions induced by the administration of inorganic iodide in massive dosage, *J. Clin. Endocrinol.* **10:** 519–531, 1950.
11. Barker, S. B.: Determination of protein-bound iodine, *J. Biol. Chem.* **173:** 715–724, 1948.
12. Leland, J. P., and Foster, G. L.: The nature of the circulating thyroid hormone, *J. Biol. Chem.* **95:** 165–179, 1932.
13. Blau, N. F.: The determination of thyroxine in the thyroid gland, *J. Biol. Chem.* **102:** 269–278, 1933.
14. Taurog, A., and Chaikoff, I. L.: The nature of the circulating thyroid hormone, *J. Biol. Chem.* **176:** 639–656, 1948.
15. Huff, S. J., and Danowski, T. S.: Unpublished studies.

ON THE MECHANISM OF IODIDE ACCUMULATION BY THE THYROID GLAND*†

JAMES B. WYNGAARDEN, M.D.,‡ JOHN B. STANBURY, M.D. AND CHARLES H. DuTOIT, M.D.‡

From the Thyroid Clinic, Massachusetts General Hospital, and the Department of Medicine, Harvard Medical School, Boston, Massachusetts

A UNIQUE property of the thyroid gland is its ability to extract the iodide ion from the blood and concentrate it; this property is retained by the thiouracilized gland *in vivo* (1, 2) and thyroid slice *in vitro* (3). In the chronically thiouracilized hypertrophied thyroid an augmentation of this accumulating ability occurs. Under these conditions this iodide exists in the gland almost entirely in the form of the iodide ion. It is not precipitated with proteins, it is dialyzable and ultrafiltrable (1, 2), and it can be oxidized to iodine by periodate (2, 3). Its potentiometric titration curve is that of the iodide ion (1).

Furthermore, animals given thiouracil and a high iodine diet may maintain a nearly normal thyroid iodine concentration, but this iodine on fractionation proves to be inorganic (4). In contrast, animals similarly treated with thiocyanate and iodine fail to accumulate inorganic iodine (4, 5). Also, thiocyanate is able to cause the quantitative discharge of iodide accumulated within the thiouracilized gland (1).

The mechanism of iodide accumulation has not yet been elucidated. It is obvious from a glance at the iodide retention curves of thiouracilized animals that the thyroid iodide is in rapid equilibrium with the plasma, and that the mechanism of iodide accumulation is a readily reversible one (6, 7). It has been suggested that iodide is bound in loose association with protein (4). Under certain circumstances homogenates prepared from hyperplastic glands contain some precipitable and nondialyzable iodide (8).

Recent work in the field of physical chemistry has shown that certain proteins, notably crystalline serum albumin, can bind small anions with

Received for publication July 25, 1951.

* Read at the Annual Meeting of the American Goiter Association, Columbus, Ohio, May 24, 1951.

This article will be included in the bound volume of the "Transactions of the American Goiter Association" published by Charles C Thomas, Publisher, which will be available for sale early in 1952.

† Aided by grants from Parke, Davis and Company, Detroit, Michigan, by an Institutional Grant from the American Cancer Society, by the Atomic Energy Commission, and by the H. N. C. gift of Harvard University.

‡ Dalton Scholar in Medicine, Massachusetts General Hospital, 1950.

variable degrees of affinity, and thus set up an unequal distribution across a semipermeable membrane (9–12). The results to be reported in this paper represent attempts to determine whether or not the peculiar affinity of the thyroid for trapping circulating iodide is dependent upon such protein anion binding. It seemed plausible that the first step in the metabolism of iodine by the thyroid might be such a mechanism developed in the thyroid cell to a high degree of specificity for iodide.

EXPERIMENTAL METHODS

Male Wistar rats averaging 180 grams in weight were fed either a low-iodine diet (13) and 0.2 per cent thiouracil in drinking water, or this diet containing 0.1 per cent propylthiouracil, for two or more weeks. They were then killed with ether, and the thyroids removed and weighed on a Roller-Smith balance. Glands were pooled and homogenized in chilled 0.1 M potassium phosphate buffer of pH 7.4. A 2-cc. sample of microscopically cell-free homogenate, generally containing 30 to 35 mg. of moist tissue per cc., was placed in a Visking 8/32 cellophane bag containing a small Pyrex bead, and the bag was then placed in a 1.2×10 cm. test tube containing 12.5 cc. of buffer and known amounts of radioiodide, with or without additional inert sodium iodide. Serum cap seals were applied, and the test tubes were inverted inside stoppered 2.4×20 cm. tubes. These were then mounted in a motor driven rack which inverted continuously every eight seconds for forty-eight to seventy-two hours at 2°C. The two phases were separated after dialysis and iodide concentration was determined by counting with an end-window Geiger-Müller counter. It was assumed that counting differences were due to protein rather than to lipid binding, since bound iodide was not soluble in carbon tetrachloride. Gibbs-Donnon factors were ignored, since in rather concentrated electrolyte solutions their contribution is negligible. Bound iodide was expressed in net counts per minute, or in micrograms per mg. of thyroid protein, or per gram of moist thyroid tissue, and normalized to 1 microcurie of radioiodine (RaI) per tube. Nonthyroidal tissues were treated in a similar manner. Serum was diluted with buffer to protein concentrations approximating those of thyroid homogenates. Protein concentrations were determined by the method of Folin and Denis (14). Iodide determinations were performed by a modification of the Barker (15) method.

In most studies the final iodide concentration ranged from 0.5 to 1.0 microgram per cent, and the radioiodide content from 0.6 to 1.2 microcuries per 14.5 cc. of solution.

The protein concentrations of thyroid averaged 13.4 per cent of the moist weight. The iodine concentrations of this thyroid tissue ranged from 0.04 to 0.11 microgram per mg. of protein, depending upon the duration

of pretreatment. Those of normal thyroid ranged from 5.0 to 8.5 micrograms per mg. of protein. For liver they were 0.000 for iodine-depleted rats, and 0.007 microgram per mg. of protein for normal rats.

Other methods used in this study, such as those employed in defining the characteristics of binding under various conditions, in determining reversibility and precipitability of bound iodide, and in identifying the state of the bound iodide, are self explanatory in the text.

The data to be presented refer to studies with hyperplastic iodine-depleted thyroid tissue except where otherwise specified.

RESULTS

Binding: Homogenized thyroid protein consistently bound radioiodide across the cellophane membrane. The bound component diminished slightly with prolonged dialysis. Most dialyses were continued for forty-eight to seventy-two hours. The iodide concentration increment not un-

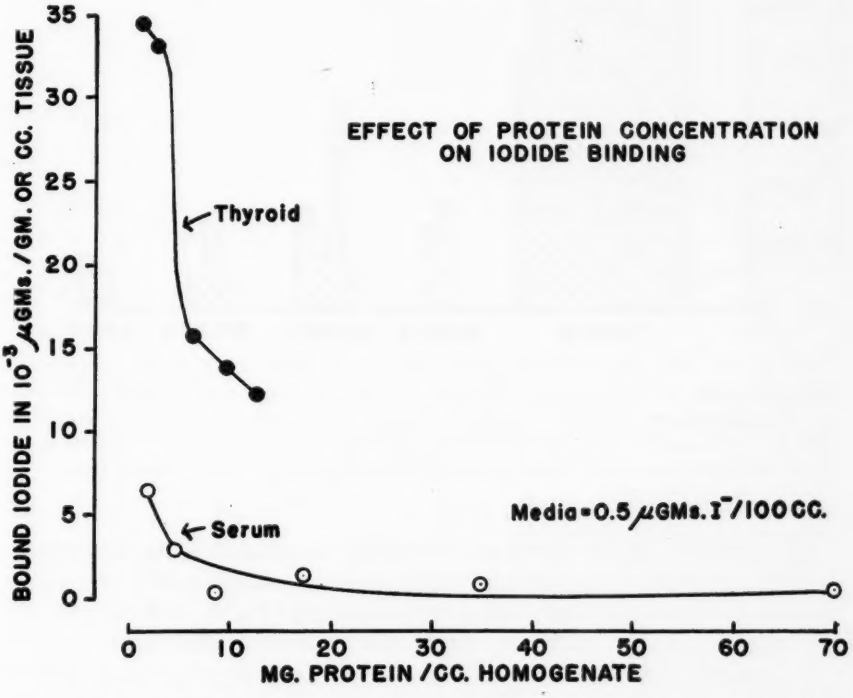


FIG. 1. Effect of serial dilutions of thyroid homogenate and of serum upon their capacity to bind iodide, in a concentration of 0.5 microgram of iodide per 100 cc. of media.

commonly reached 200 to 250 per cent. The magnitude of iodide binding per gram of moist thyroid or per cc. of serum increased markedly with dilution; at high concentrations of protein it was of a large order for thyroid compared with that of serum (Fig. 1). Thus, at a protein concentration of approximately 12 mg. of homogenate per cc., thyroid binding exceeded that of serum by about tenfold, and quite possibly this difference would be accentuated at physiologic protein concentrations of thyroid.

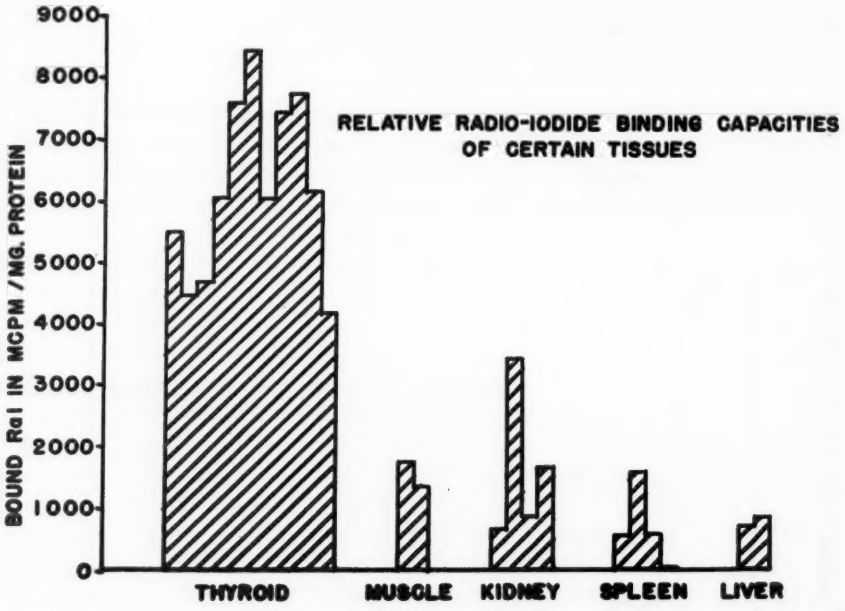


FIG. 2. The limit of significance of binding is about 600 CPM* per mg. of protein, at the homogenate concentrations employed in this study (30 mg. of moist tissue per cc.) No correction was made for the slight variation in specific activity arising from distribution of radioiodide in varying concentrations of tissue iodide. Any such correction would only accentuate the difference between thyroidal and nonthyroidal tissues.

* Counts per minute.

Nonthyroidal tissues bound small amounts of radioiodide, but this binding was generally of such a low order that the counting differences approached the limits of significance of the method (Fig. 2). Studies of nonthyroidal tissues included liver, spleen, kidney, muscle, and serum.

It appears, therefore, that cell-free thyroid protein possesses a unique capacity for binding iodide. This capacity seems to be independent of its own iodine concentration, for it was found that both homogenized normal and hyperplastic thyroid tissues bound iodide quantitatively about equally,

TABLE 1. BINDING OF IODIDE BY TISSUES OF VARYING IODINE CONCENTRATION

Tissue	Homogenate concentration (mg. protein/cc.)	Tissue iodine (μGm./mg. protein)	Bound iodide, (10^{-5} μGm./mg. protein)	Binding gradient, per cent
Thyroid	4.7	0.053	152.7 153.1	179 180
Liver	4.8	0.000	8.3 3.7	8 3
Liver + diiodotyrosine	7.8	0.195	2.7 3.5	4 5

Media iodide = 0.5 μGm./100 cc.

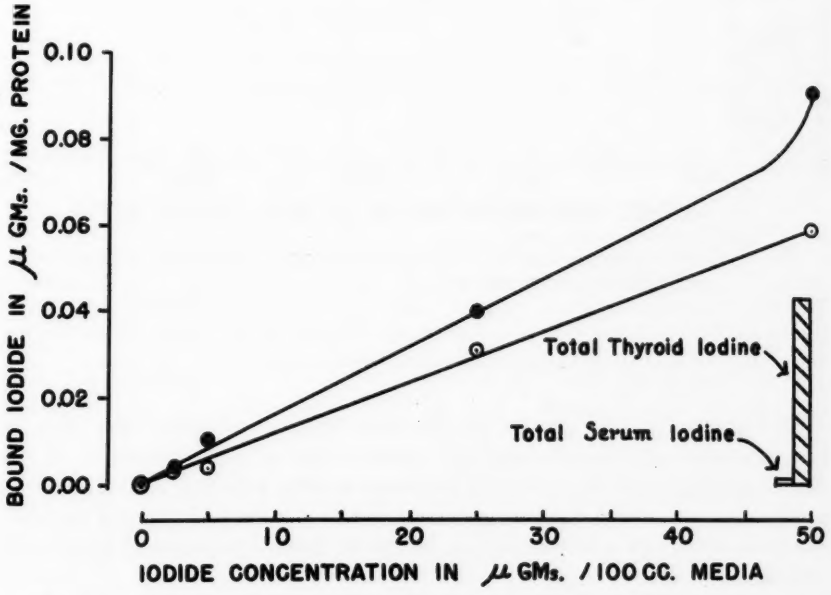


FIG. 3. Quantitative binding of iodide by thyroid and serum protein at increasing concentrations of iodide in the media. Note that both thyroid and serum bind in excess of their original total iodine concentrations, at high levels of iodide.

despite the fact that the tissue iodine concentration of the former was two hundred times that of the latter. Moreover, iodide binding, which exceeded the original iodine concentration of the tissue by more than twofold, was demonstrated at high concentrations of iodide. Furthermore, when liver homogenate was fortified with added diiodotyrosine so as to bring its final iodine concentration above that of thyroid, it bound no better than did the original liver (Table 1).

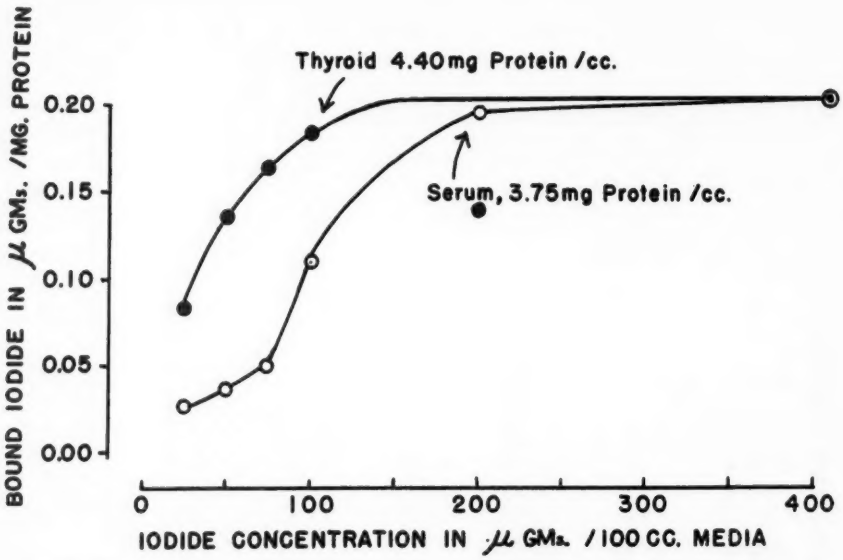


FIG. 4. A continuation of the study of quantitative iodide binding at high iodide concentrations, using different tissue preparations from those used in the study of Figure 3. The serum binding curves of Figures 3 and 4 are continuous; the thyroid binding curves are not, thus suggesting quantitatively different thyroid responses in two rat groups to pretreatment with thiouracil and low-iodine diet.

In Figures 3 and 4 are shown the quantitative iodide-binding data of homogenized hyperplastic thyroid protein and of serum protein, at increasing iodide concentrations of constant specific activity. Maximal binding is approximately 0.2 microgram of iodide per mg. of thyroid protein, and this occurs at a concentration of 150 to 200 micrograms of iodide per 100 cc. of media.

Heat-treated thyroid homogenate bound no iodide, but this may indicate tissue denaturation rather than specific enzymatic destruction.

The cellophane bag itself bound less than 5 per cent of the iodide present.

Inhibition of binding: Thiocyanate and propylthiouracil completely in-

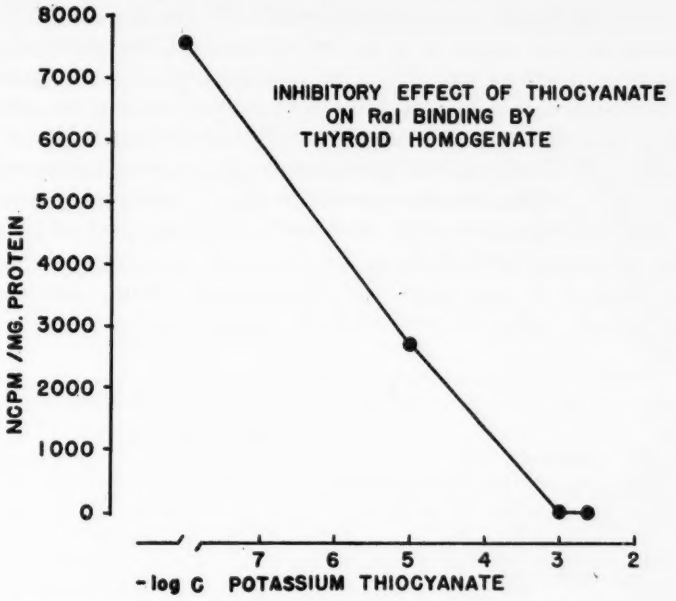


FIG. 5. The initial point represents iodide binding in the absence of any thiocyanate. Concentration of thiocyanate in moles. This study was made without carrier iodide.

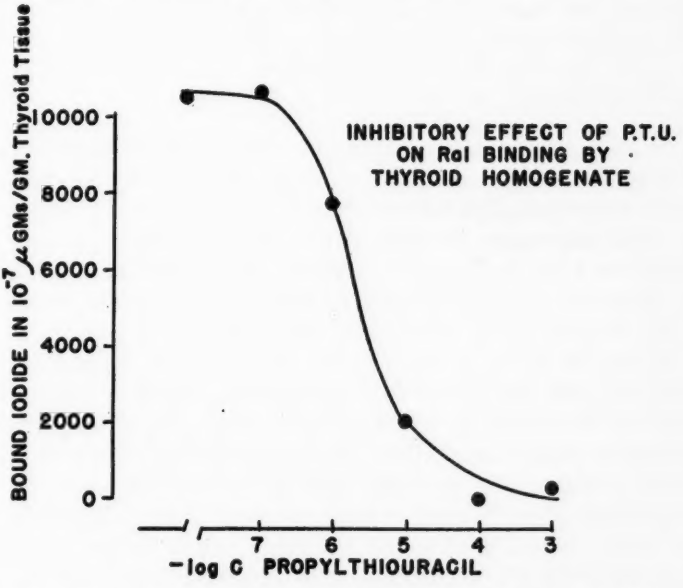


FIG. 6. Control value again represents binding in the absence of inhibitor.

hibited binding when present in appropriate concentrations (Figs. 5 and 6).

Reversibility: The relatively irreversible nature of thyroid binding was demonstrated in several ways: 1) Dilution of homogenate one hundredfold and reconstitution to a small volume by means of vacuum ultrafiltration through a collodion-coated alundum candle was attended by no loss of bound iodide. 2) Dialysis of the homogenate against running tap water for from one to forty-three days gave variable results, although generally the thyroid binding appeared to be irreversible, whereas that of serum was partially reversible. Table 2 shows the results of a study involving dilute thyroid and liver homogenates and half-strength serum, and represents the maximal reversibility observed in 17 thyroid binding experiments. In

TABLE 2. REVERSIBILITY OF HOMOGENATE-BOUND RADIOIODIDE

Tissue	Homogenate concentration in mg./cc.	Protein concentration in mg./cc.	Bound iodide in CPM* per mg. protein		Reversibility in per cent
			Initial	After 4 days' dialysis	
Thyroid	30	3.5	3700	1205	67
			3844	1979	49
Liver	30	4.0	390	51	87
			484	31	93
Serum	—	38.0	146	65	55
			171	27	84

* Counts per minute.

12, no significant reversibility was found; in 3, it was less than 28 per cent. In contrast, 45 to 85 per cent of the serum bound iodide dialyzed away in from one to four days. 3) Dialysis of the thyroid (or serum) iodide complex against serial exchanges of sodium iodide, potassium thiocyanate or sodium thiosulfate for four days caused no elution of thyroid bound iodide except for 4 and 11 per cent when dialyzed against inert iodide. Elution from serum was 39 per cent in thiocyanate and 6 per cent in iodide.

Precipitability: Four methods indicated that both thyroid and serum bound iodide were largely protein precipitable. Table 3 shows that the general order of efficiency of precipitant is zinc hydroxide > trichloracetic acid > acetone > heat coagulation. The precipitability of iodide bound by thyroid homogenate and by serum appears to be about equal.

Identification of the nature of bound iodide: After exhaustive dialysis against water, 25 per cent of the remaining bound iodide was soluble in trichloracetic acid. When 3 samples of bound iodide were fractionated into various components by the methods of Taurog and Chaikoff (16), only

3 to 8 per cent behaved like thyroxine. However, the remaining butanol-soluble fraction contained nonthyroxine-like molecules including, for example, firmly associated inorganic iodide split off from protein during alkaline hydrolysis, monoiodotyrosine, and diiodotyrosine. Thus, 92 to 97 per cent of the homogenate-bound, precipitable iodide was really unidentified by this method.

In order to establish the nature and state of the bound iodide, a butanol extract of the acidified hydrolysate was chromatographed on filter paper

TABLE 3. PRECIPITABILITY OF HOMOGENATE-BOUND RADIOIODIDE

Tissue	Precipitant	Bound iodide in CPM* per mg. protein	Precipitated iodide in CPM per mg. protein	Precipitability in per cent	
					Avg.
Thyroid	Trichloracetic acid	11,366	7,402	65	71
		8,409	4,686	56	
		756,867	562,934	74	
		427,453	381,757	89	
	Somogyi's reagent	6,029	5,662	94	78
		440,976	272,907	62	
	Acetone	211,836	126,434	60	62
		259,874	165,633	64	
	Heat coagulation	210,418	94,135	45	40
		235,855	82,899	35	
Serum	Trichloracetic acid	2,555	2,266	89	89
	Acetone	255,991	144,891	57	76
		266,560	255,364	96	
	Heat coagulation	63,998	23,871	37	31
		144,533	34,110	24	

* Counts per minute.

using the butanol-acetic acid-water, ascending unidimensional technique of Taurog, Tong, and Chaikoff (17). The great majority of the radioiodide appeared consistently in the inorganic iodide band (Fig. 7), though small amounts of activity were generally present in the monoiodotyrosine band and traces in the diiodotyrosine and thyroxine bands. This predominating band corresponded exactly in location with that of iodide trapped *in vivo* by rats with propylthiouracil-treated hyperplastic thyroid glands. There was no corresponding ninhydrin band. Moreover, in a single analysis by

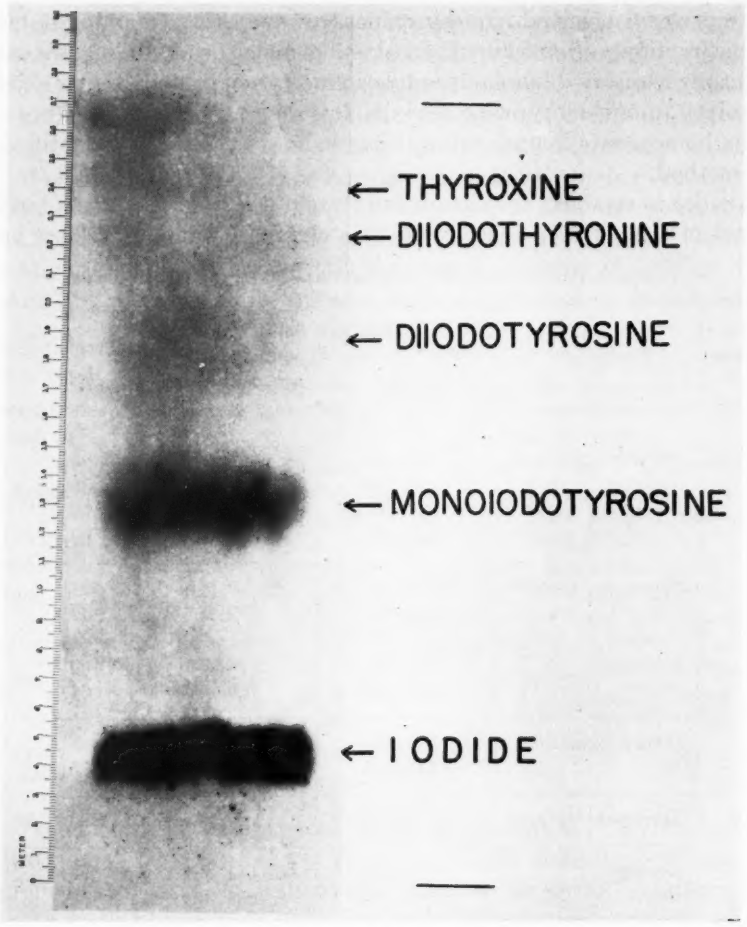


FIG. 7. Radioautograph of paper chromatogram made from butanol extract of hydrolyzed thyroid homogenate after iodide binding. The predominant radioactivity behaved like inorganic iodide.

starch column chromatography (18) the predominant activity was found in the inorganic iodide fraction.[1]

DISCUSSION

Any explanation brought forth regarding the iodide trapping mechanism will have to explain the following characteristic *in vivo* phenomena (2): 1) The thyroid iodide comes rapidly into equilibrium with plasma, but

[1] We are indebted to Miss Shirley Barry for this determination.

may be 250 or more times greater in concentration. 2) Thyroid iodide is not precipitable with trichloracetic acid or with Somogyi's reagent. 3) Thyroid iodide is completely dialyzable and ultrafiltrable. 4) The uptake by the goitrous gland is inhibited by thiocyanate, and 5) Iodide concentrated within the blocked gland can be rapidly flushed out with thiocyanate.

In the present study thyroid homogenate has revealed an unquestionable ability to bind iodide, significantly exceeding that of nonthyroidal tissues, including serum. For the following reasons it is unlikely that exchange will account for this phenomenon: 1) At 2°C. and pH 7.4 virtually no exchange should occur (19). 2) The maximal bound iodide exceeded the original iodine concentration of the thyroid tissue by more than twofold, and of serum by more than fiftyfold; and binding by liver homogenate was not enhanced by artificial fortification with organic iodine. 3) The great predominance of inorganic iodide determined by chromatographic identification is inconsistent with exchange. Hydrolysis of thyroid containing organic radioiodine does not result in the release of comparable quantities of inorganic iodide (17). 4) In the normal animal, precipitability of organic iodine is virtually complete, using trichloracetic acid (7) or Somogyi's reagent (20). The nonprecipitable fraction of bound iodide is not explainable on the basis of exchange. 5) Binding is markedly reduced in certain buffers at pH 5.5 to 6.6, where exchange should occur more readily than in alkaline phosphate.

To what degree then do the phenomena observed resemble *in vivo* accumulation by the thyroid? First of all, the magnitude of iodide accumulation is adequate. When the protein-binding data of Figures 3 and 4 are replotted in terms of grams of wet tissue and cc. of media, concentration gradients of 50 to 1 are obtained for thyroid over its media, at low iodide levels (Fig. 8). This compares very favorably with *in vivo* accumulation gradients of 250 to 1 in the propylthiouracilized hyperplastic gland, or of 25 to 1 in the acutely blocked normal gland and in the blocked sheep slice (3). At higher concentrations of homogenate tissue, gradients fall somewhat, but even at wet weight concentrations of 120 mg. per cc. they are 15 to 1.

The degrees of irreversibility and precipitability of this bound iodide are far in excess of those demonstrated for iodide trapped *in vivo*. Moreover, in our experiments, thiocyanate was ineffective in eluting bound iodide, and propylthiouracil inhibited its binding. These inconsistencies indicate that the binding demonstrated is not explanatory of the *in vivo* accumulation mechanism. Salter (5), however, has postulated two types of inorganic iodide binding by thyroid, the one a loose association easily reversible, the other a more intimate binding in which the iodide is nondialyzable, heat coagulable and acetone precipitable. The data herein reported are consist-

ent with these observations, though they do not, of course, indicate that such a union occurs physiologically. The binding observed is, however, infirm enough to allow for occasional partial reversibility on dialysis, for incomplete precipitability with several agents, and for complete emancipation upon hydrolysis.

The tenacity of the binding is unusual for ionic association, although not necessarily inconsistent. For example, iron, though remaining in the cat-

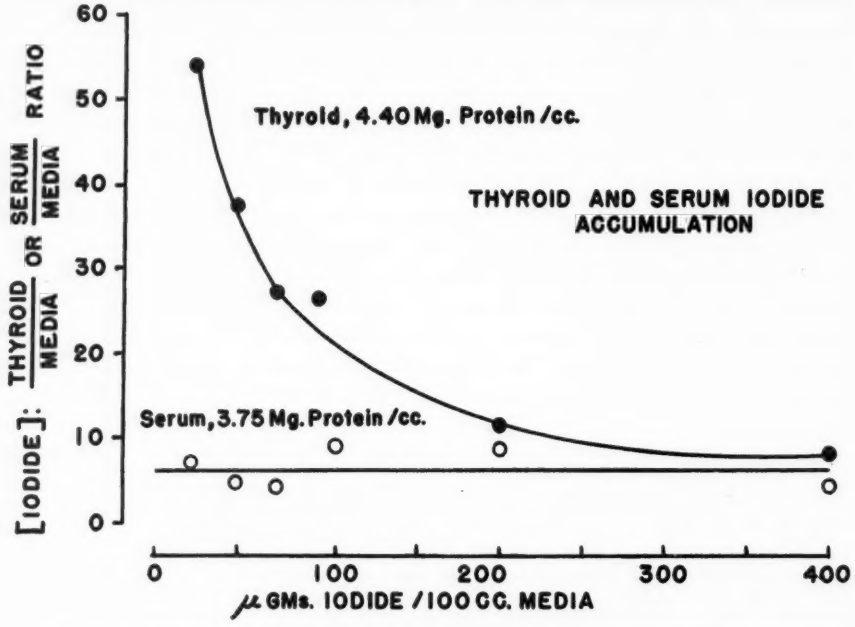


FIG. 8. A replotting of the data of Figure 4 in terms of tissue-media concentration ratios. Bound iodide was recalculated as micrograms per Gm. of moist thyroid or per cc. of serum, and compared with iodide concentrations per cc. of media.

ionic state, is irreversibly bound by B_1-pseudoglobulin at neutral pH as far as dialysis is concerned (21), and bound thiocyanate may resist ultrafiltration to a degree (22). Thus, it is possible that the binding observed represents a *protein-anion complex* of a high degree of tenacity.

A second explanation would be that the present binding occurs in consequence of an *oxidative* step. It is believed that it is an oxidative step in the metabolism of iodide which is inhibited by propylthiouracil, and also by large concentrations of thiocyanate (5). It is conceivable that binding to some unknown substance, occurring after an oxidative step, would explain several features of this study, namely the data regarding precipitability, irreversibility, lack of elution by thiocyanate, and inhibition of binding by

thiocyanate and by propylthiouracil. The chromatographic data might fit this possibility also, since it is unknown at present whether or not elemental iodine released by hydrolysis would react with sodium hydroxide to form iodide and hypoiodite, or what the stability and motility of the latter compound would be in the chromatograph solvent. Thus it is possible that the experiments described have demonstrated a divorcement of the oxidative binding step from the iodide accumulation step of thyroid iodine metabolism—in a sense, the converse of the demonstration made possible by thiouracil. Further work is necessary before either of these possible explanations can be accepted.

The demonstration of a detectable amount of radioactivity within the iodinated amino acids naturally raises the question of whether or not the complete synthesis of thyroxine can occur *in vitro* in an acellular preparation of thyroid protein, starting with inorganic iodide. It must be pointed out, however, that conditions not unfavorable for exchange were present in both chromatographic procedures, namely, an acid phase, room temperature, and adequate time. Thus it would be unwise to interpret this finding as proof that such synthesis can occur, although neither is this possibility ruled out.

CONCLUSIONS

1. Homogenized thyroid protein will consistently bind iodide, and its binding capacity greatly exceeds that of serum, liver, muscle, kidney, and spleen homogenates.
2. This binding can be inhibited by thiocyanate and by propylthiouracil.
3. The iodide bound by thyroid is largely nondiffusible and protein-precipitable. It cannot be eluted with thiocyanate, thiosulfate or iodide to any significant extent.
4. During alkaline hydrolysis this bound iodide is set free and thus appears as inorganic iodide in paper and starch column chromatography.
5. Two possible explanations for these phenomena are discussed: a) The formation of a protein-anion complex of firm association. b) The formation of an oxidative binding in a system able to by-pass the initial iodide accumulation step.

Acknowledgment

The helpful criticism of Drs. I. Herbert Scheinberg and Douglas Riggs is gratefully acknowledged. It is a pleasure also to acknowledge the technical assistance of Miss Alice Hedge, and the stimulating interest of Dr. J. H. Means.

REFERENCES

1. VANDERLAAN, J. E., and VANDERLAAN, W. P.: The iodide concentrating mechanism of the rat thyroid, and its inhibition by thiocyanate, *Endocrinology* **40:** 403, 1947.
2. TAUROG, A.; CHAIKOFF, I. L., and FELLER, D. D.: The mechanism of iodine con-

centration by the thyroid gland: its non-organic iodine-binding capacity in the normal and propylthiouracil-treated rat, *J. Biol. Chem.* **171:** 189, 1947.

3. Franklin, A. L.; Chaikoff, I. L., and Lerner, S. R.: The influence of goitrogenic substances on the conversion in vitro of inorganic iodide to thyroxine and diiodotyrosine by thyroid tissue with radioactive iodine as indicator, *J. Biol. Chem.* **153:** 151, 1944.
4. Salter, W. T.; Cortell, R. E., and McKay, E. A.: Goitrogenic agents and thyroidal iodine: their pharmacodynamic interplay upon thyroid function, *J. Pharmacol. & Exper. Therap.* **85:** 310, 1945.
5. Wolff, J.; Chaikoff, I. L.; Taurog, A., and Rubin, L.: The disturbance in iodine metabolism produced by thiocyanate: the mechanism of its goitrogenic action with radioactive iodine as indicator, *Endocrinology* **39:** 140, 1946.
6. VanderLaan, W. P., and Bissell, A.: Effects of propylthiouracil and of potassium thiocyanate on the uptake of iodine by the thyroid gland of the rat, *Endocrinology* **39:** 157, 1946.
7. Chaikoff, I. L., and Taurog, A.: Studies on the formation of organically-bound iodine compounds in the thyroid gland and their appearance in plasma as shown by the use of radioactive iodine, *Ann. New York Acad. Sc.* **50:** 377, 1949.
8. Salter, W. T.: The metabolic circuit of the thyroid hormone, *Ann. New York Acad. Sc.* **50:** 358, 1949.
9. Scatchard, G., and Black, E. S.: The effect of salts on the isoionic and isoelectric points of proteins, *J. Physical & Colloid Chem.* **53:** 88, 1949.
10. Scatchard, G.; Scheinberg, I. H., and Armstrong, S. H., Jr.: Physical chemistry of protein solutions. IV. The combination of human serum albumin with chloride ion, *J. Am. Chem. Soc.* **72:** 535, 1950.
11. Scatchard, G.; Scheinberg, I. H., and Armstrong, S. H., Jr.: Physical chemistry of protein solutions. V. The combination of human serum albumin with thiocyanate ion, *J. Am. Chem. Soc.* **72:** 540, 1950.
12. Klotz, I. M.: The nature of some ion-protein complexes, *Symposia on Quantitative Biology, Cold Spring Harbor, N. Y.* **14:** 97, 1950.
13. Remington, R. E.: Improved growth in rats on iodine deficient diets, *J. Nutrition* **13:** 223, 1937.
14. Folin, O., and Denis, W.: Nitrogen determinations by direct nesslerization. I. Total nitrogen in urine, *J. Biol. Chem.* **26:** 473, 1916.
15. Barker, S. B.: Determination of protein-bound iodine, *J. Biol. Chem.* **173:** 715, 1948.
16. Taurog, A., and Chaikoff, I. L.: The metabolic interrelations of thyroxine and diiodotyrosine in the thyroid glands as shown by a study of their specific activity-time relations in rats injected with radioactive iodine, *J. Biol. Chem.* **169:** 49, 1947.
17. Taurog, A.; Tong, W., and Chaikoff, I. L.: The monoiodotyrosine content of the thyroid gland, *J. Biol. Chem.* **184:** 83, 1950.
18. Barry, S., and Dobyns, B.: Unpublished method.
19. Miller, W. H.; Anderson, G. W.; Madison, R. K., and Salley, D. J.: Exchange reactions of diiodotyrosine, *Science* **100:** 340, 1944.
20. McGinty, D. A.: Iodine absorption and utilization under influence of certain goitrogens, *Ann. New York Acad. Sc.* **50:** 403, 1949.
21. Surgenor, D. M.; Koechlin, B. A., and Strong, L. E.: Chemical, clinical, and immunological studies on the products of human plasma fractionation. XXXVII. The metal-combining globulin of human plasma, *J. Clin. Investigation* **28:** 73, 1949.
2. Rosenbaum, J. D., and Lavietes, P. H.: Lipoid-thiocyanate in serum, *J. Biol. Chem.* **131:** 663, 1939.

THE USE OF THIOURACIL IN THE TREATMENT OF METASTATIC CARCINOMA OF THE THYROID WITH RADIOIODINE*†

J. E. RALL, M.D., WADE N. MILLER, M.D., CHARLES G. FOSTER, M.D., WENDELL C. PEACOCK, PH.D., AND RULON W. RAWSON, M.D.

From the Department of Clinical Investigation of the Sloan-Kettering Institute and Memorial Center, New York, and the Medical Department of Brookhaven National Laboratories, Upton, Long Island, N. Y.

INTRODUCTION

DURING the past five years a systematic attempt has been made at Memorial Center to induce in cancers of the thyroid a capacity to concentrate radioiodine like that possessed by normal thyroid tissue (1, 2). Total thyroidectomy, the administration of thyrotropic hormone and thiouracil have been the measures used to influence the cancers. Long continued pretreatment with thiouracil has been the most effective means of increasing iodine concentration by cancers of the thyroid. This report is a summary of the results obtained so far with this method.

METHODS

The methods of standardization of radioiodine, and its measurement in blood, urine and *in vivo* have been described previously (3). The estimation of the absolute amount of radioiodine in the thyroid by these methods is technically possible to a probable error of about 15 per cent. Because it has been impossible to estimate accurately the absolute amount of radioiodine in one or more large, deeply situated metastases, the quantity of radioiodine excreted in the urine in four days has been used as an index of the amount concentrated in the metastases. The relative concentration of radioiodine by metastatic areas has been determined by counting with

Received for publication July 25, 1951.

* Read at the Annual Meeting of the American Goiter Association, Columbus, Ohio, May 26, 1951.

This article will be included in the bound volume of the "Transactions of the American Goiter Association" published by Charles C Thomas, Publisher, which will be available for sale early in 1952.

† This work has been supported by an institutional research grant from the American Cancer Society, by a grant-in-aid from the American Cancer Society, a grant from the National Cancer Institute of the U. S. Public Health Service, by an institutional grant from the Damon Runyon Memorial Fund for Cancer Research and a contract from the Atomic Energy Commission #AT(30-1)910.

TABLE 1. COMPARISON OF THE EFFECT OF THIOURACIL, THYROIDECTOMY AND THYROID-STIMULATING HORMONE ON THE CONCENTRATION OF RADIOIODINE BY CANCERS OF THE THYROID

Agent used to stimulate I^{131} uptake	Total number of patients	Number of patients in whom tumor uptake increased	Per cent of patients in whom tumor uptake increased
Thiouracil	30	21	70.0%
Thyroidectomy	35	21	60.0%
TSH	16	5	31.3%

the Geiger-Mueller tube at skin contact. An increased uptake has been interpreted when the counting rate over the involved area increased by a factor of 5. Thiouracil has been administered in doses of from 1.0 to 1.5 grams per day. In all patients total thyroidectomy, either surgically or with radioiodine, preceded the administration of thiouracil. Tracer doses of radioiodine were given at approximately six-week intervals throughout the course of thiouracil administration. In all instances the administration of thiouracil was discontinued forty-eight hours prior to the radioiodine tracer and was not resumed until the tracer study had been completed.

RESULTS

Table 1 shows the effects of thyroidectomy, TSH and thiouracil on the concentration of radioiodine by metastatic cancers of the thyroid. There was sufficient concentration of radioiodine by the cancer to permit the

TABLE 2. EFFECT OF THIOURACIL ON THE UPTAKE OF I^{131} BY METASTATIC CARCINOMA OF THE THYROID

Pathologic diagnosis	Increased uptake	No effect
Metastasizing struma	3	1
Follicular carcinoma with or without alveolar and solid components	9	2
Papillary and follicular carcinoma with solid or alveolar components	5	1
Papillary carcinoma	2	1
Solid carcinoma	1	2
Hürthle cell carcinoma (follicular metastases)	1	2
	21 (70.0%)	9 (30.0%)

Comparison of the pathologic diagnoses in patients given thiouracil to increase the concentration of radioiodine by the cancers. A biopsy of the metastases in 1 case of primary Hürthle cell carcinoma showed a follicular structure. This case showed an increased uptake of radioiodine in the metastases after pretreatment with thiouracil.

administration of a therapeutic amount without resorting to at least one of these procedures, in only 2 out of 20 cases.

It is apparent that of the three procedures, thiouracil was the most effective agent for increasing radioiodine uptake by metastatic lesions. In all cases, however, thyroidectomy was performed prior to the administration of thiouracil, so it is in some cases difficult to ascertain whether the

TABLE 3. DIAGNOSES—PATIENTS TREATED WITH THERAPEUTIC DOSES OF I^{131}

Metastasizing struma	3
Follicular carcinoma with or without alveolar and solid components	9
Papillary and follicular carcinoma with solid or alveolar components	5
Papillary carcinoma	1
Solid carcinoma	1
Hürthle cell carcinoma (follicular metastases)	1

increased uptake was a late effect of thyroidectomy or a direct effect of thiouracil. Table 2 shows the pathologic diagnoses in those cases in which thiouracil caused an increase in the uptake of radioiodine. Table 3 shows the pathologic diagnoses in 20 patients who received radioiodine in thera-

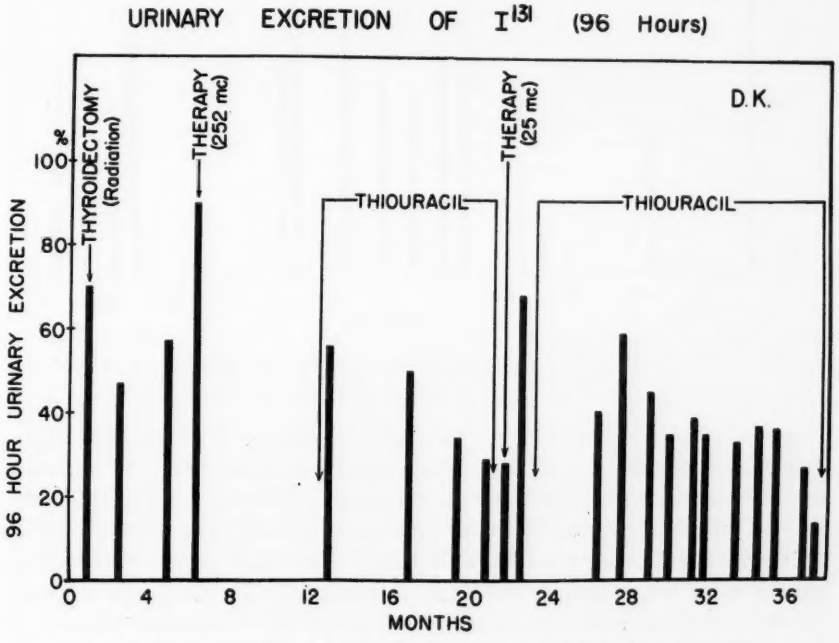


FIG. 1. Urinary excretion of multiple tracer doses of radioiodine in a patient (D. K.) with cancer of the thyroid and pulmonary metastases.

peutic amounts. It is significant that in all but one of those patients successfully prepared with thiouracil, there was some element of follicle formation in the cancer. It is also significant that only 2 of the 20 patients eventually treated with radioiodine failed to show a follicular component in the cancer.

Three patterns of response to thiouracil have been observed. The first has been a complete failure of thiouracil to influence the uptake of radioiodine. This has been observed in 9 patients. In 3 of these, however, the

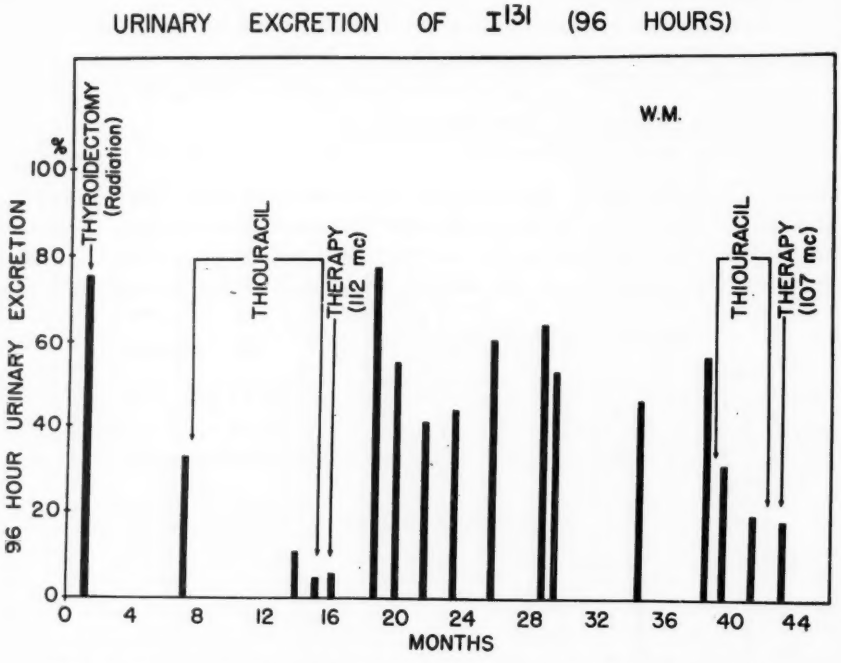


FIG. 2. Urinary excretion of multiple tracer doses of radioiodine in a patient (W. M.) with cancer of the thyroid and pulmonary metastases.

thiouracil was administered for two months or less. It is possible that a longer period of treatment would have produced some effect. It is of considerable interest that 5 of the 9 patients who did not respond to thiouracil had no follicular elements in their cancers (Table 2). The second pattern of response is shown by D. K., a 15-year-old girl with papillary, follicular and alveolar carcinoma of the thyroid, metastatic to the lungs. Figure 1 shows 96-hour urinary excretions of radioiodine at intervals before and after varying periods of pretreatment with thiouracil. It is apparent that there was a gradual increase in avidity of the metastatic lesions for radio-

iodine. The radioactivity over the metastases, as determined by *in vivo* counting, correlated inversely with the urinary excretion of the tracer dose.

The third pattern of response has been the abrupt development of an extremely high uptake of radioiodine by the carcinomas. This is shown in the case of W. M., a 15-year-old boy with metastasizing struma and pulmonary metastases. Figure 2 shows the 96-hour urinary excretion of many tracer doses of radioiodine before and after a therapeutic dose. It is difficult to be sure whether total thyroidectomy or thiouracil, or both, caused the striking decrease in urinary excretion of tracer doses of radioiodine seen in the first sixteen months of observation. After the therapeutic dose of 112 millicuries of radioiodine, however, there was no significant change in the urinary excretions of tracer doses of radioiodine for a period of twenty months. Within two months after the institution of therapy with thiouracil a marked change was noted, so that the urinary excretion in ninety-six hours of a tracer dose of radioiodine had decreased from 60 per cent to 20 per cent. A causal relationship between this change and the thiouracil seems likely.

TABLE 4. MEAN 96-HOUR URINARY EXCRETION OF BOTH THERAPEUTIC AND PRETREATMENT TRACER DOSES OF RADIOIODINE IN PATIENTS WITH CANCER OF THE THYROID

Number of doses	Mean dose	96-Hour mean urinary excretion	
		Tracer	Therapy
	Doses prior to December 1948		
20	148 mc.	63%	65%
	Doses subsequent to December 1948		
17	96 mc.	31%	33%

Table 4 shows data to indicate the trend in treatment of metastatic cancer of the thyroid at Memorial Center. It is apparent that prior to December, 1948, over 60 per cent of the average therapeutic dose of radioiodine was excreted in ninety-six hours, whereas after this time, only approximately 30 per cent of the average therapeutic dose was excreted in ninety-six hours. This date represents the introduction of the use of large amounts of thiouracil in the pretreatment of these patients.

Sixteen patients have been treated with large doses of radioiodine after preparation with thiouracil. Three of these have shown a diminution in the pulmonary metastases demonstrated radiographically. Figure 3 shows the chest roentgenograms of a boy of 15 before and after the administration of 112 millicuries of radioiodine. It is apparent that there has been a

dramatic decrease in the pulmonary metastases in this boy. It is obviously too early to evaluate the late result of this therapy, for he was treated only two and a half years ago. We have not observed recalcification in osteolytic metastases, but in 3 patients have seen arrest of growth of the skeletal metastases. There has been some objective improvement noted in 9 of the 16 patients. Relief of pain has been noted in 6. In all patients, some improvement was noted.

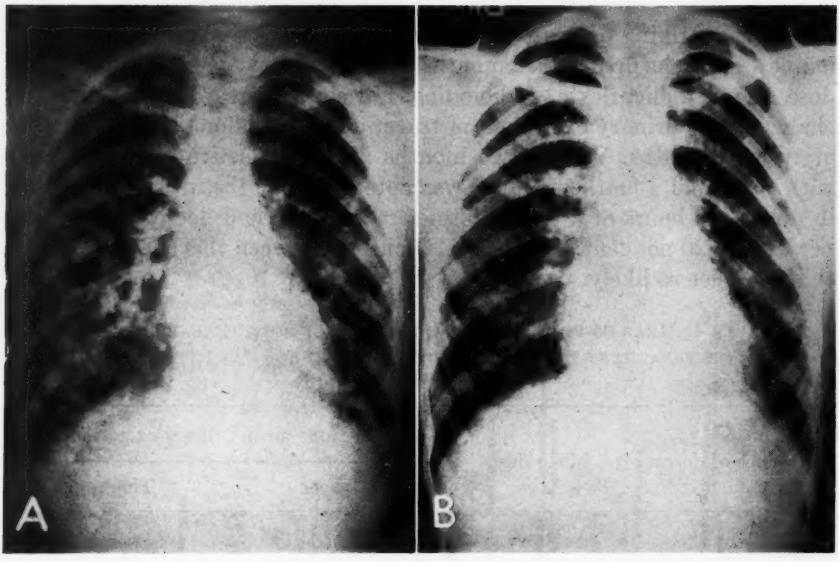


FIG. 3. Chest roentgenograms of patient W.M. with carcinoma of the thyroid, metastatic to the lungs. The picture on the left was taken January 19, 1948. A therapeutic dose of I^{131} (112 millicuries) was given on December 29, 1948. The picture on the right was taken July 6, 1950.

Toxic effects observed as a result of the administration of thiouracil were less than might have been expected. No cases of agranulocytosis developed in these patients, although a leukopenia of 3,000 cells per cu. mm. has been observed in many of them. In 3 patients psychoses developed during thiouracil treatment; in 2, the psychoses cleared up as soon as the myxedema was treated with desiccated thyroid. One psychosis has persisted and has been ascribed by psychiatrists to previous deep-seated emotional disturbances. One patient showed urticaria of such severity that it was necessary to discontinue the thiouracil.

DISCUSSION

Thiouracil itself has been used in all these cases. Although 6-n-propyl-

thiouracil has been used only a few times, the impression has been gained that it is much less effective.

In 5 patients, preparation with thiouracil was so effective that less than 15 per cent of the dose was excreted in the urine in ninety-six hours. The explanation of this dramatically high uptake of radioiodine requires some consideration. At least three possibilities arise. One, as a result of having taken thiouracil for a long period of time, the patient is in a state of iodine depletion so that the avidity for iodine of any functioning thyroid tissue, cancerous or not, is extremely high. Analogy may be made to animals given a low-iodine diet; the uptake of radioiodine by the thyroid may be increased from 10 to 50 per cent within as little as a month of treatment with such a diet (4). A second explanation is that thiouracil, by causing myxedema in these patients, has stimulated the pituitary to put out an increased amount of thyroid-stimulating hormone, which in turn increases the avidity of the thyroid tissue, cancerous or normal, for iodine. A third explanation is that these large doses of thiouracil have augmented the action of the patient's own circulating thyroid-stimulating hormone. The *in vitro* and *in vivo* augmentation of the action of TSH by thiouracil has been shown previously (5, 6). There is no evidence to indicate which one or combination of these explanations obtains.

Possible serious objections to the long-term pretreatment of patients with thiouracil are: 1) the patient may die before therapeutic doses of radioiodine are administered, and 2) the tumors may increase in size so that although more radioiodine is picked up by the tumors, the actual concentration of radioiodine per gram of tumor decreases. The first objection rarely obtains because most cancers of the thyroid are very slow-growing. The second objection is difficult to refute unequivocally because it is impossible to determine precisely the mass of tumor tissue in, for example, lungs, and its change during the course of pretreatment with thiouracil. Serial x-ray plates of roentgenographically demonstrable metastases, however, have failed to show marked change during thiouracil pretreatment.

SUMMARY

It has been possible by a combination of total thyroidectomy and prolonged thiouracil treatment to induce collection of radioiodine by 21 of 35 metastatic cancers of the thyroid.

It has been possible as a result of this preparation to administer 27 therapeutic doses of radioiodine to 16 patients.

REFERENCES

1. Marinelli, L. D.; Trunnell, J. B.; Hill, R. F., and Foote, F. W.: Factors involved in the experimental therapy of metastatic thyroid cancer with I^{131}; a preliminary report, *Radiology* **51**: 553–557 (Oct.) 1948.

2. TRUNNELL, J. B.; MARINELLI, L. D.; DUFFY, B. J., JR.; HILL, R.; PEACOCK, W., and RAWSON, R. W.: The treatment of metastatic thyroid cancer with radioactive iodine: credits and debits, *J. Clin. Endocrinol.* **9**: 1138–1152 (Nov.) 1949.
3. HILL, R. F.; HINE, G. J., and MARINELLI, L. D.: Quantitative determination of γ radiation in biological research, *Am. J. Roentgenol.* **63**: 160–169 (Feb.) 1950.
4. MONEY, W. L.: Personal communication.
5. RAWSON, RULON W.: Physiological reactions of the thyroid stimulating hormone, *Ann. New York Acad. Sc.* **50**: 491–506 (Jan.) 1949.
6. ALBERT, A.; RAWSON, R. W.; MERRILL, P.; LENNON, B., and RIDDELL, C. B.: The effect of goitrogenic and other reducing agents on inactivated thyrotropic hormone extract, *Endocrinology* **40**: 299–302 (May) 1947.

MALIGNANT THYROID TUMORS OCCURRING IN THE MOUSE AFTER PROLONGED HORMONAL IMBALANCE DURING THE INGESTION OF THIOURACIL*

HAROLD P. MORRIS, PH.D., ALBERT J. DALTON, PH.D. AND CELIA DUBNIK GREEN, B.A.

From the National Cancer Institute, National Institutes of Health, Federal Security Agency, Public Health Service, Bethesda 14, Maryland

INTRODUCTION

IT IS generally recognized that the surgical removal of an endocrine gland will affect the secretion of other endocrine glands. It would be expected, too, that a chemical agent capable of specifically blocking the secretion of a single endocrine gland would also alter the normal hormonal balance. Experiments on mice have been in progress for over seven years in our laboratories on the effects of long continued chemically produced hormonal imbalance (1). The goitrogenic drug, thiouracil, in these experiments has been the chemical agent used to interfere with the secretions of the thyroid gland.

The expected hypertrophy and hyperplasia of the thyroid gland of mice treated for prolonged periods with thiouracil were accompanied by structural changes in other endocrine glands notably the adrenal, pituitary, testes and ovary (2, 3). Young mice ingesting thiouracil continuously also ceased increasing in body weight at an early age. Estrus was inhibited or completely suppressed. Mammary gland development was arrested, and mammary-tumor formation in susceptible mice was reduced to a very low incidence or to zero (3, 4).

The theoretical mechanism advanced to explain these results features the creation of a hormonal imbalance initiated by the inability of the thyroid gland to secrete thyroxine while under the influence of a goitrogen; followed by a compensatory over-secretion of thyrotropin (TSH) by the anterior lobe of the hypophysis with a concomitant undersecretion of other tropic hormones.

In spite of the extreme hypertrophy and hyperplasia of the thyroid

Received for publication July 25, 1951.

* *Second Honorable Mention, 1951 Van Meter Prize Award of the American Goiter Association.*

This article will be included in the bound volume of the "Transactions of the American Goiter Association" published by Charles C Thomas, Publisher, which will be available for sale early in 1952.

glands of mice ingesting thiouracil, with glands weighing from 3 to 30 times more than those of control mice, no evidence of neoplasia was found in the original thyroids (2).

A considerable proportion of the lungs of the strain C3H mice, in which thyroids had been maintained in this hyperplastic state for periods exceeding twelve months, grossly exhibited numerous superficial pearly gray nodules identified histologically as thyroid tissue (2). Because of the presence of thyroid tissue in blood vessels of the thyroids of the mice ingesting thiouracil as noted by Dalton, Morris, and Dubnik (2) and by Gorbman (5), and because of the presence of endothelium surrounding the nodules of thyroid tissue in the lungs (2) it was believed that such nodules represented true metastases from thyroid glands, even though the metastatic nodules were very similar histologically to the thyroids of the animals in which they were found.

This paper presents the results of a series of experiments dealing with the transplantability and growth potential of hyperplastic and metastatic thyroid tissue, the development of autonomous malignant tumors from such hyperplastic tissue after a series of transplant generations and a brief description of the histologic and some physiologic characteristics of two tumor lines.

METHOD

It was essential to prolong the period of hyperplasia beyond the lifetime of any one mouse in order to test adequately the hypothesis that prolonged TSH stimulation would eventually result in further alteration progressing to neoplasia and possibly even malignancy. It was also necessary to provide conditions under which thyroid tissue could be exposed to excessive TSH stimulation for very long periods under the most favorable conditions which could be devised.

The body fluids of highly inbred mice of the same strain (C3H) in which hyperplastic thyroids were present seemed to offer the most nearly homologous fluid in which to grow thyroid tissue. In order to keep the tissues under continuous TSH stimulation indefinitely, the tissues were transferred by means of a sterile trocar at frequent intervals to young hosts which had ingested a diet of natural foodstuffs containing thiouracil[1] for a few weeks before the transfer.

In the early stages of the experiments very little tissue was available, so that only a few animals could be used. Whenever possible a small bit of tissue was preserved in Zenker-formol and stained with hematoxylin and eosin at each transfer so that a serial histologic record would be obtained.

[1] The thiouracil used was generously supplied by Lederle Laboratories, Pearl River, N. Y., through the courtesy of Dr. S. M. Hardy.

This was not always possible at the time of some of the early transfers because in these, all of the tissue available was needed for transplantation.

RESULTS

The initial transfer was made from a thyroid gland of a strain C3H mouse that had ingested a thiouracil-containing diet for eighteen months. The hyperplastic thyroid tissue of this mouse was implanted subcutaneously into 4 young mice of the same strain. In all 4 cases the transplanted tissue developed a vascular bed very rapidly and grew well and each of the 4 sublines was carried for several generations. The detailed description which follows has been confined to but 2 of the 4 recipient hosts and their descendants.

The subcutaneous implant in host #1 was carried as generation #1 for nine months, at which time it was transferred to other young hosts (generation #2). After four months in these hosts it was again transplanted (generation #3). In a third generation host after three months, autopsy revealed a lung nodule which had the glistening, pearly gray gross appear-

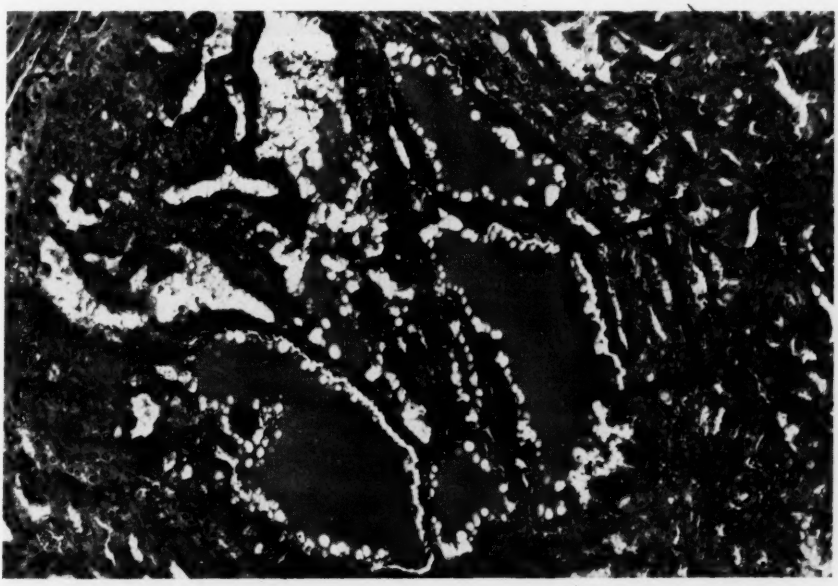


FIG. 1. Adenomatous area in the thyroid of the mouse found to possess a pulmonary nodule. From this area, on transplantation, tumor line #1 developed. The adenoma is different from any other seen previously in the thyroids of C3H mice ingesting thiouracil, and contained simple columnar cells lining large follicles containing acidophilic colloid. The arrangement of the cells is obviously different from that of the surrounding hyperplastic thyroid tissue. ×150.

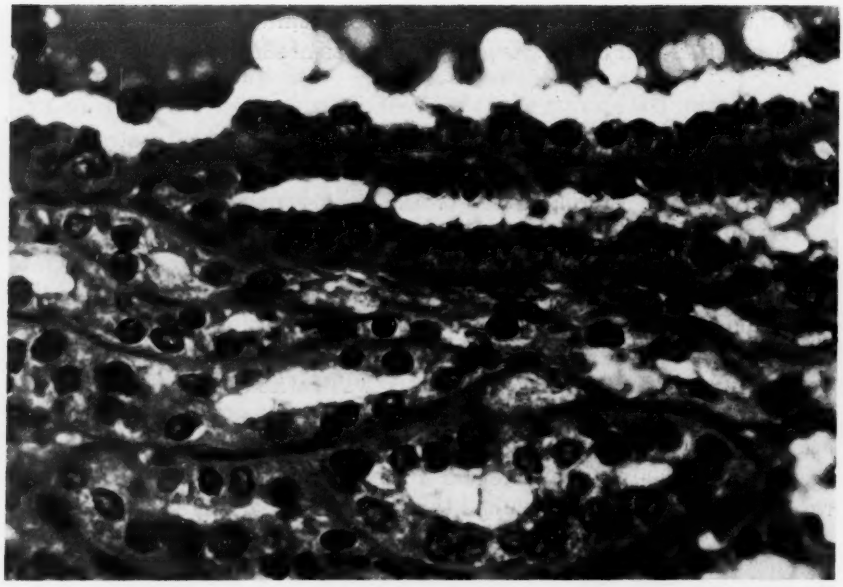


FIG. 2. Cell detail at margin of the adenomatous area shown in Figure 1. The adenoma cells are smaller and their nuclei are more hyperchromatic (upper part of field) than those of the typical hyperplastic thyroid tissue (lower part of field). ×800.

ance of the nodules which previous experience had associated with the presence of metastatic thyroid tissue. This nodule was transplanted in its entirety subcutaneously to a young host ingesting thiouracil. The morphologic evidence for this metastatic nodule having consisted of thyroid tissue is based on studies of subsequent implant generations and particularly on the similarity between the histologic structure of these implants and an adenomatous area found in the thyroid of host #1. This tumor line at first grew only in mice ingesting thiouracil but in the seventh generation was found to grow readily in normal mice. It has been designated tumor line #1.

The second of the recipient hosts referred to above carried part of the same original thyroid subcutaneously also for nine months; the implant was then transferred to other mice in exactly the same way through 10 transplant generations. This other line will be referred to in this paper as tumor line #4. Line #4 was dependent on thiouracil for continued growth up through the eighth generation. In the ninth generation, a transplant grew in 1 mouse out of 7 receiving the stock diet without thiouracil, showing that at this time complete autonomy had been attained. However, in line #4 the best growth and highest percentage of takes still continued to be obtained in mice ingesting thiouracil.

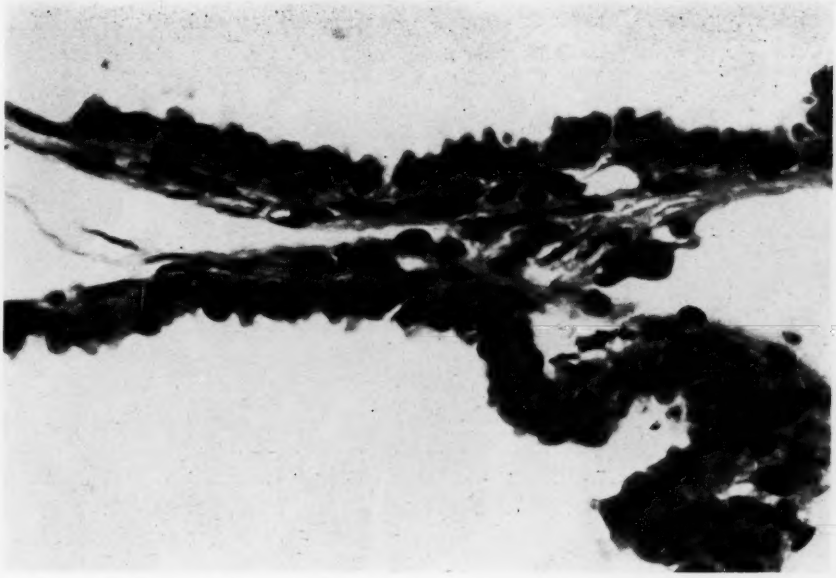


FIG. 3. Cell detail at the margin of the cystic area in the fourth generation transplant, showing a cell type and arrangement similar to that in the adenomatous area in Figure 2. ×800.

As indicated above, tumor line #1 resulted from the transplantation of a pulmonary nodule in a mouse which itself was carrying a subcutaneous implant of thyroid tissue. In spite of its somewhat uncertain origin (as a metastasis either from the host thyroid or from the subcutaneous implant) this tumor line was chosen for detailed histologic study because, as will be shown subsequently, it was representative of a tumor type which retained very little of normal thyroid function. No material was available from the first generation transplant for histologic study, since it was all used as transplanting material. All of the later generations of transplants however, are fundamentally similar histologically and the individual cells of these transplants are similar to an adenomatous area in the thyroid of the animal from which the lung nodule was taken and dissimilar to the subcutaneous implant which was growing in the original host. Figure 1 shows this adenomatous area surrounded by hyperplastic thyroid tissue. The follicles of the adenoma are lined by a layer of simple columnar epithelium and are the only follicles in the sections of this thyroid gland which were found to contain colloid. Figure 2 shows the detail of the relatively small adenoma cells with their hyperchromatic nuclei. The fourth generation transplant, as did all of the early transplant generations, developed as a cystic vesicle containing a central mass of tissue. Figure 3 shows an

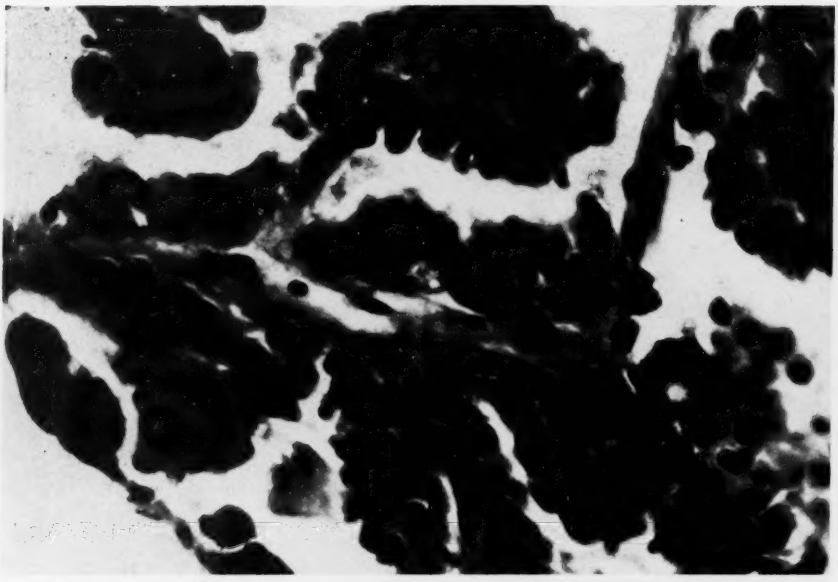


FIG. 4. Cell detail in the area of the central mass in a fourth generation transplant, showing a condensed, cord-like arrangement of the cells. ×800.

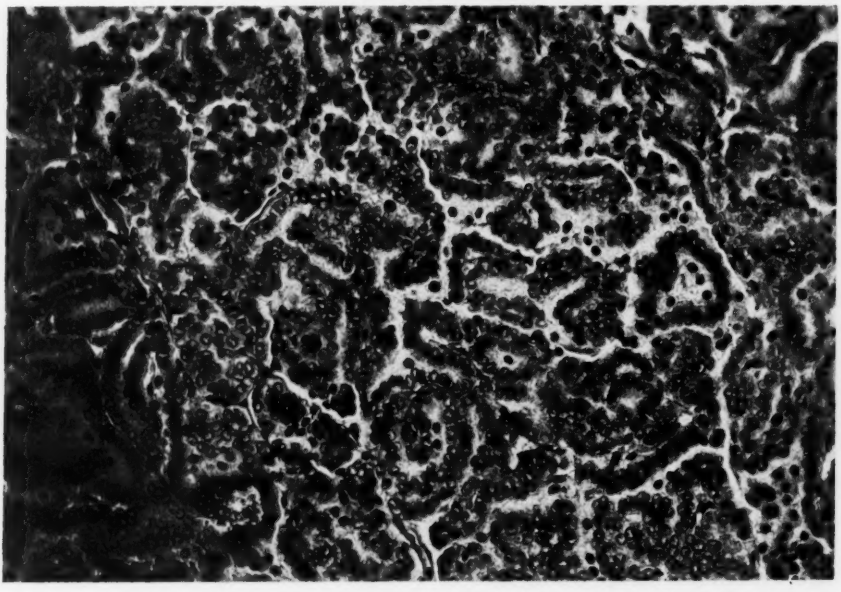


FIG. 5. Section from a seventh generation transplant, showing the tortuous cord-like arrangement of the cells. ×300.

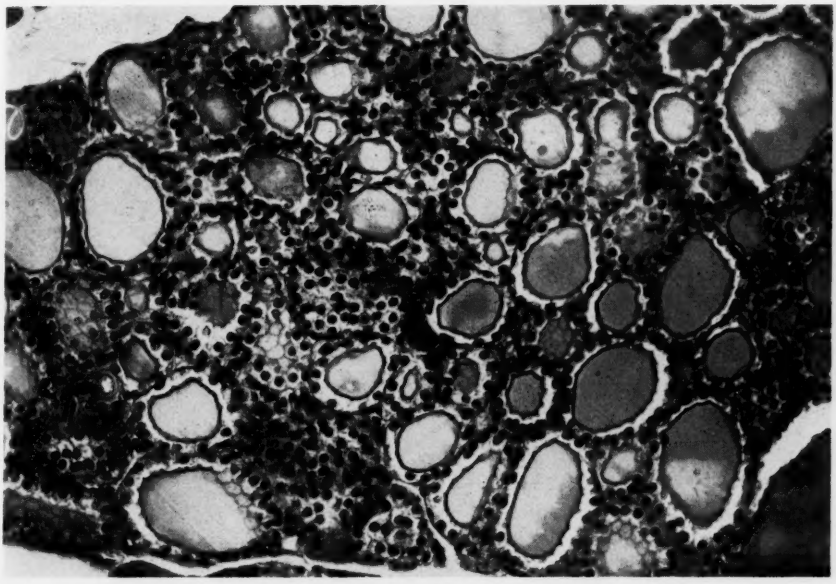


FIG. 6. Thyroid of the host carrying a ninth generation transplant. This animal had not received thiouracil and the thyroid shows no deviation from the normal picture. ×300.

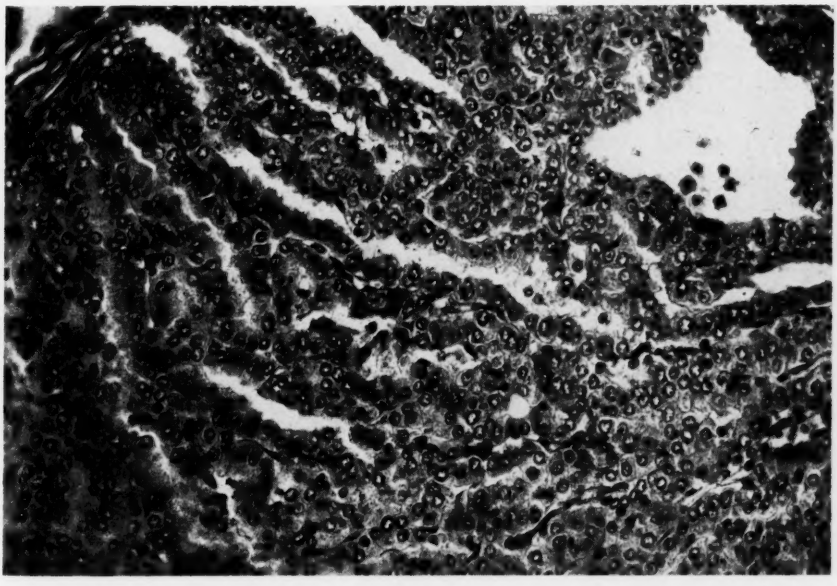


FIG. 7. Cell arrangement of tumor line #1 in the ninth generation transplant. ×300.

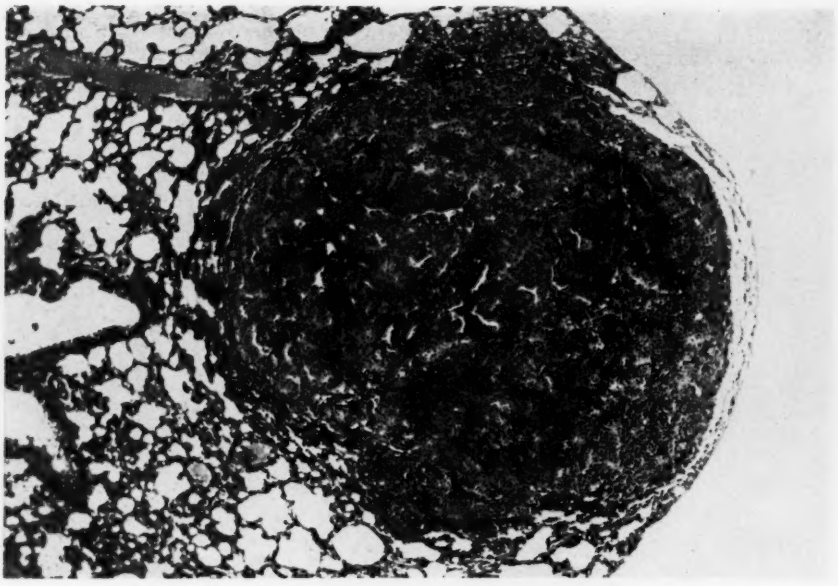


FIG. 8. Low-power view of a pulmonary metastasis from a subcutaneous implant of the ninth generation of tumor line #1. ×75.

area at the margin of the cyst with a cell type and cell arrangement similar to that of the adenoma, and Figure 4, from the central tissue mass, shows a similar type of cell arranged in closely packed, simple cords. In all of the generations of transplants, the cells are arranged in these simple cords which run an extremely tortuous course. This arrangement was already explicit in the fourth generation (Fig. 4) and became characteristic of the whole tumor by the seventh generation (Fig. 5). In the seventh generation, the tumor was found to grow readily in mice not receiving thiouracil. In the ninth generation, not only did the tumor grow autonomously but numerous pulmonary metastases developed as well. Figure 6 shows the normal appearance of the thyroid of the host in this particular case and Figure 7 shows the histologic pattern of the tumor cells of the subcutaneous implant of this generation. Figure 8 shows a pulmonary metastasis from this subcutaneous implant and Figure 9, in more detail, shows its similarity in cell type and arrangement to the subcutaneous implant. Figure 10 shows that the characteristic cord-like arrangement of the tumor cells was continued in the eleventh generation.

Tumor line #4, which arose as described previously, was chosen for detailed histologic analysis because, in contrast to tumor line #1, it represented a tumor type with a considerable capacity to assimilate and

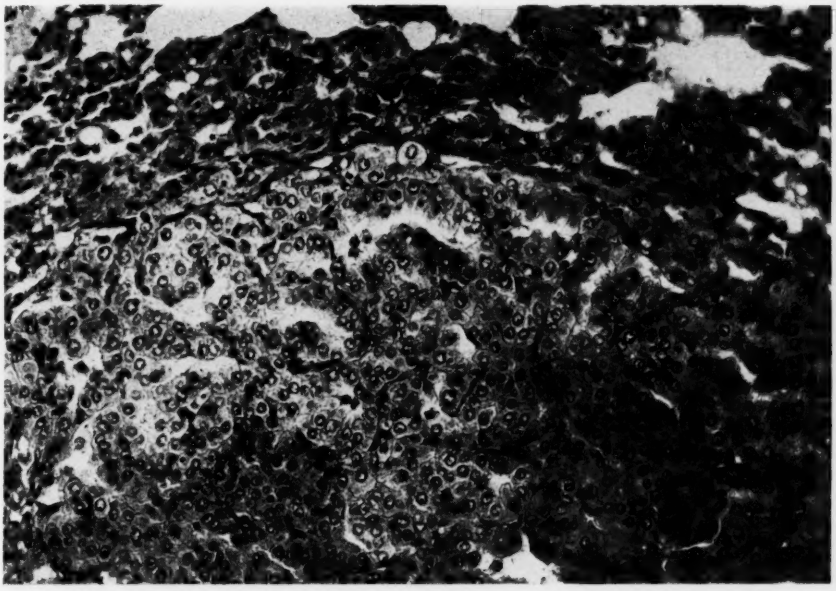


FIG. 9. High-power view of part of the pulmonary metastasis shown in Figure 8, illustrating a cell arrangement similar to that of the subcutaneous implant in the same animal (Fig. 7). ×300.

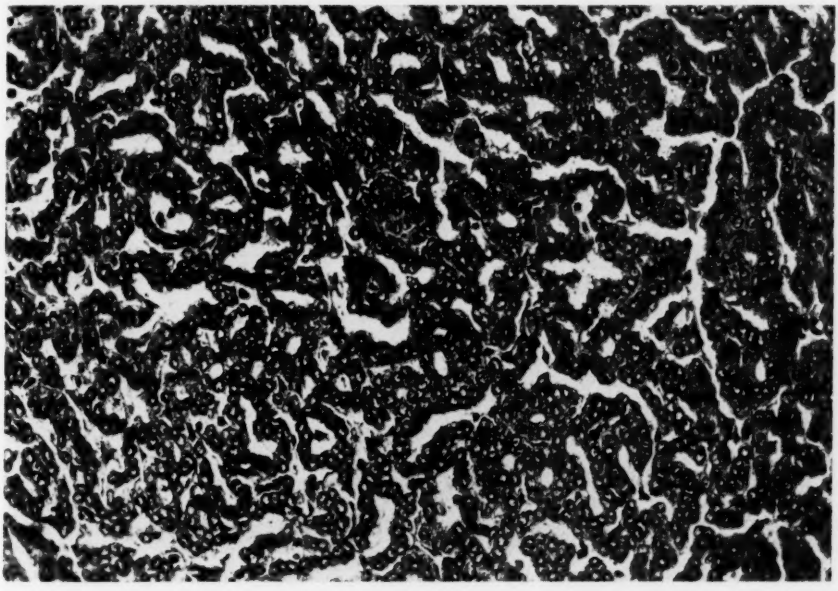


FIG. 10. Subcutaneous implant of tumor line #1 in the eleventh generation, showing a continuation of the characteristic cell arrangement as in previous generations. ×300.

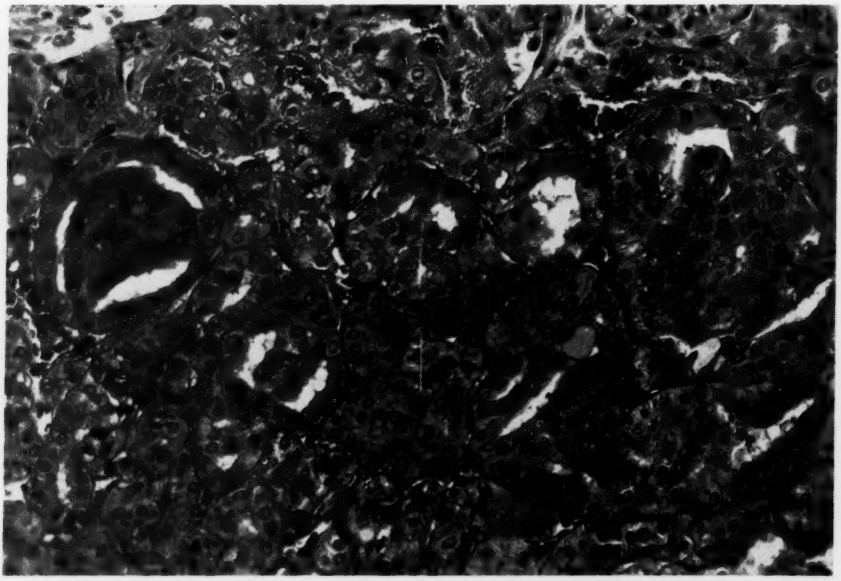


FIG. 11. Subcutaneous implant of the fifth generation of tumor line #4, showing a cell type and arrangement similar to typical hyperplastic thyroid tissue (Fig. 2). ×300.

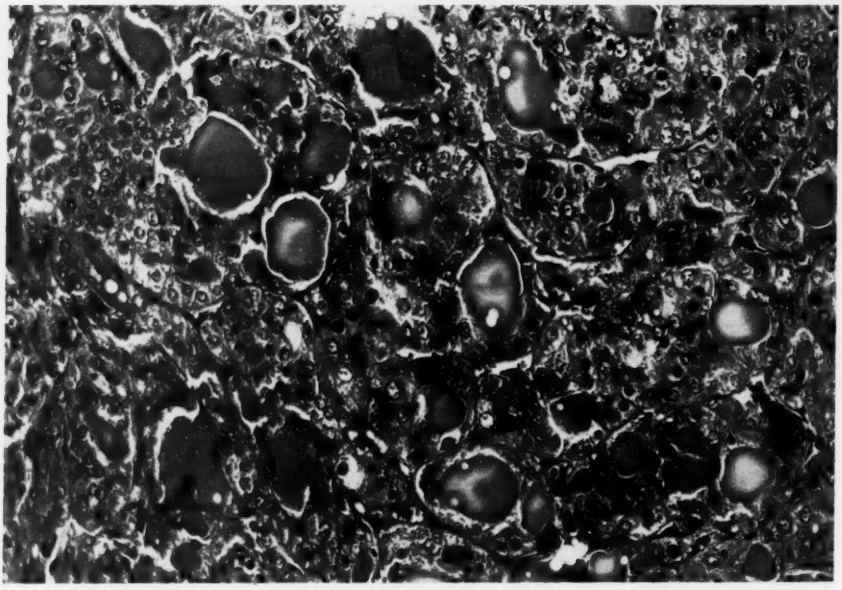


FIG. 12. Subcutaneous implant of the ninth generation of tumor line #4, grown in a mouse ingesting thiouracil, showing a similarity in cell type and arrangement to that of the fifth generation, except that acidophilic colloid is present in the follicles. ×300.

store iodide. The cells of this tumor from the first possessed a cell type and arrangement very similar to typical hyperplastic thyroid tissue (compare the hyperplastic tissue in Fig. 2 with the tissue in Fig. 11). The cells are relatively large and possess vesicular, relatively hypochromatic nuclei. The essential difference between tumor line #4 and the hyperplastic thyroid tissue is the presence in the tumor of follicles containing moderately acidophilic colloid. No difference in histologic structure could be detected in this tumor when, in the ninth generation, the tumor was grown

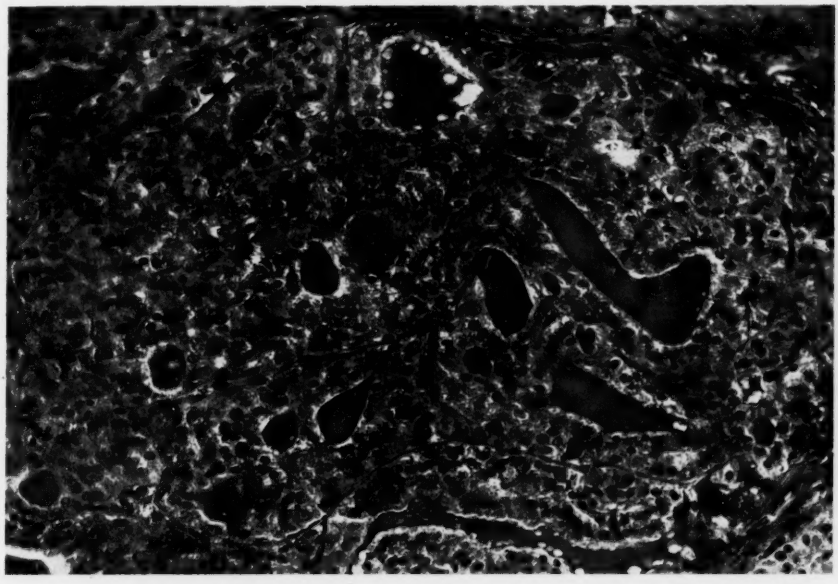


FIG. 13. Subcutaneous implant of the ninth generation of tumor line #4 grown in a mouse not receiving thiouracil, showing a detailed structure essentially similar to that shown in Figure 12. ×300.

in some mice ingesting thiouracil (Fig. 12) and in other mice not ingesting thiouracil (Fig. 13).

Thyroid and tumor function

Wollman, Morris and Green (7) found that there was a great difference in the ability of these two tumor lines to function as thyroid tissue. They calculated the rate of the percentile uptake of I^{131} per mg. of tumor and compared this with the rate per mg. of thyroid tissue of the host. This ratio gives a comparison of the functional capacity of the two tissues in the same animal where the tissues would be exposed to the same hormonal

environment. Using animals in transplant generation 7, the concentrating power of the host thyroid in tumor line #1 was found to be 1,300 times that of the tumor, whereas for tumor line #4 it was only 2.16 times that of the tumor (Table 1). Hence, even in the case of the most thyroactive tumor (which at this time was still dependent on thiouracil for continued growth) the tumor concentrated iodide on the basis of unit weight of tissue only half as well as did the thyroid in the same animal. The tumors grow to be much larger than the host thyroid so that their total iodine uptake may actually amount to much more than the pickup in the thyroid. This is illustrated in Table 2, in which the iodine pickup by the thyroid is com-

TABLE 1. AVERAGE PERCENTILE UPTAKE OF I^{131} BY THYROIDS AND TUMORS PER MG. OF TISSUE

Mouse tumor line	Time after injection (hrs.)	A	B	Ratio A/B
		% I^{131}/mg. of thyroid	% I^{131}/mg. of tumor	
1	5	2.60	0.0024	1300.00
4	18	1.38	0.65	2.16

TABLE 2. AVERAGE PERCENTILE UPTAKE OF I^{131} BY HOST THYROIDS AND TUMORS

Mouse tumor line	Time after injection (hrs.)	A	B	Ratio A/B
		I^{131} in thyroid, %	I^{131} in tumor, %	
1	5	25.7	0.21	202.00
4	18	12.1	44.40	0.31

pared with the uptake by the tumor, showing that in tumor line #1 the thyroid took up approximately 200 times more I^{131} than the tumor, whereas in tumor line #4 the tumor took up three times as much I^{131} as the thyroid gland of the host.

Using the ability of a tissue to convert iodide into thyroxine as another criterion of functional activity, it was found at eighteen hours after injection that in tumor line #4 about 11 per cent of the I^{131} taken up was in the thyroxine fraction. Moreover, the ratio of the fraction I^{131} as thyroxine in the tumor to that of thyroxine in the thyroid was 1:1, indicating that at this time tumor line #4 was about as effective as the thyroid in converting iodide into thyroxine.

DISCUSSION

Several other lines of transplanted tumors other than those described in detail here, have been obtained. One line was developed from the thyroid of a mouse that ingested the carcinogen 2-acetylaminofluorene, and thiouracil simultaneously. Some implants in this subline attained complete autonomy in the third transplant generation. The histologic appearance of this subline is very similar to that of tumor line #1 described previously. It is still not clear, however, whether the carcinogen exerted a procarcinogenic effect on the hyperplastic thyroids. Another line originating from a lung nodule has been carried through four transplant generations without attaining complete autonomy. Other sublines still require TSH stimulation for progressive growth. When such stimulation is withdrawn, by removing thiouracil from the diet of the host, the implant undergoes regression.

The question of the origin of the pulmonary nodule from which tumor line #1 arose is interesting but relatively unimportant. The early generations of this tumor are similar histologically to an adenomatous area in the thyroid of the mouse in which the pulmonary nodule was found, and dissimilar to the tumor line which was growing as a subcutaneous implant in this same animal. However, the host had been ingesting thiouracil for but three months—a much shorter time than our experience indicates would result in the development of pulmonary metastases. In any case, the original host was too young (5 months of age at autopsy) to have shown either spontaneous mammary or pulmonary tumors, neither of which was found macroscopically or microscopically. Thus, regardless of its origin, tumor line #1 undoubtedly arose from thyroid tissue which had been stimulated for a relatively long period by excessive amounts of TSH as a result of thiouracil ingestion.

Bielschowsky (8) has reported that 2-acetylaminofluorene (AAF), when administered to rats simultaneously with a goitrogen, acted as an initiating or promoting agent in producing thyroid tumors. More recently Hall and Bielschowsky (9) reported that, from morphologic evidence, malignancy also appeared in the thyroid of the rats receiving only the goitrogen for one and a half years. Invasion of neighboring structures, metastases, and penetration of the capsule were used as criteria for establishing the diagnosis of malignancy. These investigators concluded from the later studies that the AAF accelerated the appearance of multiple benign adenomata, but probably the essential factor in the pathogenesis of the experimental tumors of the rat thyroid was the continuous stimulation by TSH. Money and Rawson (10, 11) consider that, in addition to the overstimulation of the thyroid by TSH, the iodine deficiency produced by the action of anti-

thyroid drugs may also be involved in the process of thyroid tumor formation in rats.

Bielschowsky *et al.* (12) have reported transplantation of hyperplastic thyroid tissues to thyroxine-deficient rats or thyroidectomized rats but they were unsuccessful in transplanting such rat thyroid tumors to normal rats, despite the presence of other morphologic signs characteristic of malignancy. The inoculated material of Bielschowsky *et al.* (12), which grew only when a state of thyroid deficiency with its consequent increased output of TSH was maintained in the host, shows that the tumors were not autonomous but could be maintained only under the same conditions of thyroid hyperplasia produced by the increased TSH stimulation responsible for the production of the original tumor.

It is believed the experiments reported herein are the first experimental evidence showing that autonomous thyroid tumors can be produced in mice after prolonged ingestion of thiouracil and presumably similarly prolonged TSH stimulation. The fact that our mouse thyroid tissues were subjected for a longer period to a continuous state of stimulation than were the rat tissues of other observers, may account for the successful development of thyroid tumors in mice transplantable to normal animals. Another factor may be the strain difference in mice in response to TSH stimulation, for it has been observed that forty C (B alb C)-strain mice fed the same thiouracil-containing diet for similar intervals of time showed numerous thyroid adenomata (13), but only one instance of thyroid metastasis to the lung. Such histologic observations support the view that possibly the entire thyroid of the C3H mouse was altered by TSH stimulation, whereas only small localized areas of the gland in the C strain were so affected (13). Previously (2) we had suggested, primarily on morphologic evidence, that the presence of thyroid tissue in the lungs of C3H mice ingesting thiouracil was possibly an instance of "benign metastasizing thyroid tissue." These latest results make it necessary for us to conclude that, in some instances at least, we were dealing with neoplastic, potentially malignant tissue.

SUMMARY

The long continued exposure of thyroid tissue to excessive amounts of thyrotropic hormone by means of serial transplantation of such tissue to mice ingesting thiouracil has resulted in the transformation of hyperplastic thyroid tissue into malignant autonomous thyroid neoplasms from which pulmonary metastases frequently develop.

The degree of autonomy and the tendency to metastasize show a correlation with the degree to which the histologic pattern of the tumors differs from that of hyperplastic thyroid tissue. A correlation has also been dem-

onstrated between their histologic picture and the relative capacity of the tumors of lines #1 and #4 to function as thyroid tissue, as indicated by their ability to assimilate iodine and to transform iodide into thyroxine.

REFERENCES

1. Morris, H. P., and Green, C. D.: The role of thiouracil in the induction, growth, and transplantability of mouse thyroid tumors, *Science* **114:** 44–46, 1951.
2. Dalton, A. J.; Morris, H. P., and Dubnik, C. S.: Morphologic changes in the organs of female C3H mice after long-term ingestion of thiourea and thiouracil, *J. Nat. Cancer Inst.* **9:** 201–223, 1948.
3. Morris, H. P.; Green, C. D., and Dalton, A. J.: The effect of ingestion of thiouracil on strain C mice, *J. Nat. Cancer Inst.* **11:** 805–815, 1951.
4. Dubnik, C. S.; Morris, H. P., and Dalton, A. J.: Inhibition of mammary-gland development and mammary-tumor formation in female C3H mice following ingestion of thiouracil, *J. Nat. Cancer Inst.* **10:** 815–841, 1950.
5. Gorbman, A.: Thyroid changes induced by prolonged feeding of thiourea, *Cancer Research* **6:** 492, 1947.
6. Morris, H. P.: Review of nutritive requirements of normal mice for growth maintenance, reproduction and lactation, *J. Nat. Cancer Inst.* **5:** 115–143, 1944.
7. Wollman, S.; Morris, H. P., and Green, C. D.: Functionality of four transplantable mouse tumors, *J. Nat. Cancer Inst.* **12:** No. 1 (Aug.) 1951.
8. Bielschowsky, F.: Tumors of the thyroid produced by 2-acetylaminofluorene and allyl-thiourea, *British J. Exper. Path.* **25:** 90–95, 1944.
9. Hall, W. H., and Bielschowsky, F.: The development of malignancy in experimentally induced adenomata of the thyroid, *British J. Cancer* **3:** 534–541, 1949.
10. Money, W. L., and Rawson, R. W.: Experimental production of thyroid tumors in male rats, *Tr. Am. A. Study Goiter* pp. 171–178, 1947.
11. Money, W. L., and Rawson, R. W.: The experimental production of thyroid tumors in the rat exposed to prolonged treatment with thiouracil, *Cancer* **3:** 321–335, 1950.
12. Bielschowsky, F.; Griesbach, W. E.; Hall, W. H.; Kennedy, T. H., and Purves, H. D.: Studies on experimental goiter: the transplantability of experimental thyroid tumors of the rat, *British J. Cancer* **3:** 541–546, 1949.
13. Dalton, A. J.; Morris, H. P.; Striebich, M. J., and Dubnik, C. S.: Histologic changes in strain C mice following long-term ingestion of thiouracil, *J. Nat. Cancer Inst.* **11:** 391–413, 1950.

RESPONSES TO RADIOACTIVE IODINE THERAPY IN HYPERTHYROIDISM, WITH SPECIAL REFERENCE TO CARDIAC PROBLEMS*†

FARAHE MALOOF, M.D.‡ AND EARLE M. CHAPMAN, M.D.

From the Thyroid Clinic and Department of Medicine of the Harvard Medical School at the Massachusetts General Hospital, Boston, Massachusetts

THE preliminary use of radioactive iodine in the diagnosis and treatment of diseases of the thyroid (1–5) stands out as one of the major achievements in medicine in the past decade. The origins and early experience with the use of this isotope have been reviewed recently (6, 7). Continued experience with this form of therapy has enabled us to observe its advantage or disadvantage in patients with varying clinical forms of hyperthyroidism. Here we wish to present data gained from treating 330 such patients with radioactive iodine and especially in treating 47 of them who had heart disease or heart symptoms as the chief clinical manifestation of their departure from health.

We seek to evaluate the usefulness of radioactive iodine in the treatment of these particular patients whose cardiac problem is the major consideration. Two objections have been raised to the use of radioactive iodine. First, that the length of time needed to control the hyperthyroidism constitutes a hazard to the patient's life; and second, that internal radiation of the thyroid causes a release of thyroid hormone and a resultant rise in the protein-bound iodine of the serum which would aggravate the existing heart disease or even precipitate thyrotoxic crisis. It has been on the basis of these fears that some physicians have given antithyroid drugs or iodide to patients soon after their therapeutic dose of radioactive iodine. We believe that this polypharmaceutical procedure has only obscured the results of the radiation therapy and as yet has yielded no precise information.

HISTORICAL REVIEW

Parry (8) in 1786, was not only the first to describe the symptoms of

Received for publication July 25, 1951.

* Read at the Annual Meeting of the American Goiter Association, Columbus, Ohio, May 24, 1951.

This article will be included in the bound volume of the "Transactions of the American Goiter Association" published by Charles C Thomas, Publisher, which will be available for sale early in 1952.

† Aided by a Grant-in-aid from the Atomic Energy Commission.

‡ Post-Doctorate Public Health Service Research Fellow of the National Cancer Institute.

exophthalmic goiter, but was the first to make note of a possibly related goiter and an enlarged heart. Trousseau (9) in 1863 believed that iodine aggravated thyrotoxicosis in most instances and hence was averse to its use. One day he gave tincture of iodine by error to a patient with thyrotoxicosis and heart failure. The patient improved; in spite of this he changed the therapy to tincture of digitalis when he discovered his error. The patient became worse. Even this failed to convince him of the therapeutic value of iodine in thyrotoxicosis.

The modern era of the interrelationship between the thyroid and heart disease may be assigned to the studies of Lahey and Hamilton (10) and also Levine (11) who pointed out the difficulty in making the diagnosis of hyperthyroidism in the presence of severe heart disease, since often the cardiac manifestations overshadow the underlying thyrotoxicosis. The usual ophthalmic findings are not present and at times it is difficult to palpate any enlargement of the thyroid. It was Lahey who proposed the term "thyrocardiac" for these particular patients.

METHODS AND MATERIAL

After careful clinical evaluation of our patients with hyperthyroidism, we obtained an assay of the protein-bound iodine of the serum (hereafter called PBI) and serial basal metabolic rates (BMR's). Tracers of radioactive iodine were given before therapy and the thyroid uptake at the end of forty-eight hours was determined (12); in many, the urinary excretion of radioactive iodine in this same period or longer was also determined; directional counting was performed in those patients suspected of having nodular goiters (13).

The heart disease was evaluated by the physician in charge of the ward and the consultants in cardiology, aided by electrocardiograms and chest roentgenograms.

Many of these patients had previous therapy such as surgery, iodine, or antithyroid drugs. Once the diagnosis of hyperthyroidism had been suggested, the patients were presented before the Thyroid Clinic for discussion, definitive studies, and decision of the treatment best suited to the individual.

The dosage of radioactive iodine for each patient is still a problem (14) but we relied on a dose of 160 microcuries of I^{131} (half-life, eight days) per estimated gram of tissue, based on an average uptake of 70 per cent.

In order to assay the biologic reaction to this form of radiation therapy we followed most patients closely and obtained the following:

1. Basal metabolic rates and serum PBI determinations on the second, fourth, eighth, sixteenth, thirty-second and sixty-fourth day after treatment.

2. Weekly *in vivo* measurements of radioactivity over the thyroid area for eight to ten weeks after therapy.
3. Consecutive 24-hour urine collections.
4. Results of tracer doses of radioactive iodine at intervals after treatment.
5. Repeated assays of the cardiac function.

From Table 1 it may be noted that none of our patients was below the age of 40. Hurxthal (15) found little, if any, evidence of cardiac damage in thyrotoxic patients under 40 years of age in whom there was no evidence of organic heart disease. Thyrotoxicosis usually occurs from four to six times as often in the female as in the male; in our small series of 47 patients the ratio is about 2 to 1. Five of the patients had nodular goiters; and in 10 the disease was recurrent after thyroidectomy. It was very difficult at times to assess accurately the duration of the thyrotoxicosis, but it appeared that the illness was of several years' duration in most of the patients.

TABLE 1. A GROUP OF 47 PATIENTS WITH PREDOMINANTLY CARDIAC MANIFESTATIONS SELECTED FROM 330 PATIENTS WITH THYROTOXICOSIS TREATED WITH RADIOACTIVE IODINE

47 Patients with Hyperthyroidism and Cardiac Symptoms	
Age—No patient under 40; all but 8 were 50 or older	
Sex—Females, 32; Males, 15	
Type of goiter	
Diffuse	32
Nodular	5
Recurrent-postop.	10
Duration of disease—several years	

In Table 2 we have attempted to classify the types of heart disease and have recorded the number of cases of congestive failure occurring in each type. Congestive failure did not preponderate in any particular type of heart disease. Auricular fibrillation was the most frequent type of arrhythmia, and it occurred more often in the patients of the 50–59 age group, in whom the heart disease and the degree of toxicity appeared to be more severe and of longer duration (Table 3). Congestive heart failure occurred in 16 of the 27 patients with auricular fibrillation (Table 3).

THE CLINICAL RESPONSE OF PATIENTS TO THERAPY

In these 47 patients with thyrotoxicosis and cardiac symptoms the response to treatment is noted in terms of interval of time, the number of doses of radioactive iodine required to obtain the response, the total

TABLE 2. THE INCIDENCE OF CONGESTIVE FAILURE AND THE TYPE OF RHYTHM IN THE DIFFERENT TYPES OF HEART DISEASE

Type of heart disease	No. of patients	Congestive failure	Rhythm		
			Auricular fibrillation	Paroxysmal tachycardia	Regular
Thyrocardiac*	12	5	8†	1	3
Hypertensive and arterioscler.	20	12	10	4	6
Coronary, with angina	8	5	3	1	4
Rheumatic	4	2	4	0	0
Type ?*	3	1	3	0	0
Total	47	25	28†	6	13

* Thyrocardiac = patients with no evidence of organic heart disease that could be assigned to the usual classification while toxic or when euthyroid. It is difficult to state that a 74-year-old patient (Case 25), classified as a thyrocardiac, had no cardiac vascular abnormalities, but Maher and Sittler (16) expressed this problem well when they concluded that "thyrotoxicosis brought to the surface latent cardiovascular lesions, which resumed their latency on the successful termination of the thyroid toxemia." Type ? = patients with definite evidence of organic heart disease, which we were unable to classify.

† One had auricular flutter.

TABLE 3. THE INCIDENCE OF AURICULAR FIBRILLATION AND CONGESTIVE HEART FAILURE IN THE DIFFERENT AGE GROUPS

Age group	No. of patients	Auricular fibrillation	Congestive failure	Auricular fibrillation with congestive failure
40–49	8	5*	5	4
50–59	16	14	6	6
60–69	11	3	8	2
70–79	11	6	5	3
80–89	1	0	1	1
Total	47	28*	25	16

* One had auricular flutter.

TABLE 4. SUMMARY OF THE DATA ON THE 47 PATIENTS WITH THYROTOXICOSIS AND CARDIAC PROBLEMS

Patient (Case No. & initials)	Age	Sex	Type of heart disease	Auricular fibrill.	Before treatment			Radioactive iodine treatment				Response of patient	Remarks
					BMR, %	Serum PBI, γ %	48-hr. Uptake of tracer by thyroid, %	Dose (mc)		48-hr. Excretion, %	Total dose (mc)		
6 M.D.	49	F	Thyrocardiac	0	+25 Recurrent	—	66.0	7.0		44.0	7.0	Euthyroid in 3 mos.	
24 E.DeC.	55	M	Thyrocardiac	+	+60	—	—	10.0	11/15/48	15.0	28.0	None	Still toxic; fibrillating slowly. No digitalis or quinidine. Very resistant in spite of good uptake. See Fig. 5b.
					+58	—	—	9.0	4/29/49	8.0		None	
					+59	14.0	100.0	9.0	10/28/49	8.0		Slight	
25 S.W.	74	F	Thyrocardiac	+	+25 Nodular	—	68.0	12.0		36.0	12.0	Euthyroid in 2 mos.	Good response of a hyperfunctioning nodule to I^{131}. See Fig. 3 and Fig. 5a.
26 A.J.	65	F	Thyrocardiac	0	+48 Recurrent	—	59.0	12.0		46.6	12.0	Euthyroid in 3 mos.	Previous KI treatment. Myxedema in 5 mos.
29 R.L.	57	F	Thyrocardiac	+	+26 Recurrent	—	—	33.0*		16.6	33.0*	Moderate	Still toxic with slow auricular fibrillation after treatment with I^{130}. BMR normal; did not reflect her disease. Died 5 yrs. after I^{130} treatment.
30 R.C.	51	F	Thyrocardiac	+	+16	15.9	85.0	9.0		7.1	9.0	Myxedema developed in 3 mos.	Paroxysmal auricular fibrillation and recurrent heart failure for 1 yr. before diagnosis made. Stopped fibrillating 2 mos. after treatment.
31 E.W.	51	F	Thyrocardiac	+	+17 Recurrent	13.9	85.0	13.3		18.1	13.3	Good	Taking propylthiouracil until 2 wks. before treatment. Local physician reported normal in 3 mos. Regular rhythm in 6 mos.
33 D.S.	56	M	Thyrocardiac	+	+33	14.8	72.0	8.0		25.0	8.0	Myxedema in 3 mos.	Stopped fibrillating in 2 mos.
37 J.M.	48	M	Thyrocardiac	+†	+38	13.0	88.0	9.0		15.3	9.0	Euthyroid in 1 mo.	Fairly rapid response. Rhythm regular except for occasional ventricular extrasystole.
40 C.P.	65	M	Thyrocardiac	0	+ 7	—	75.0	10.0		11.7	10.0	BMR normal in 2 mos.	Taking propylthiouracil until 3 days before I^{131} therapy. BMR and PBI elevated, but 48-hr. uptake and disappearance rate of iodide from the blood normal 8 mos. after therapy.‡
46 W.M.I.	45	F	Thyrocardiac	+	+50	—	—	14.8	6/13/49	9.5	40.4	No response	Subtotal thyroidectomy after 9 mos. of propylthiouracil. See text and Fig. 6.
					+56	—	78.0	12.0	9/17/49	9.0			
					+52	12.1	71.0	13.6	1/26/50	27.3§			
47 A.L.	47	F	Thyrocardiac	0	+39 Recurrent	—	88.0	14.0		10.9	14.0	—	Died 6 weeks after treatment of pneumonia and heart failure. Still toxic.
1 F.S.	60	F	Hypertensive	0	+42	—	—	60.0*	5/30/46	—	60.0*	—	Not seen for 3 yrs. after 2nd dose, at which time she was euthyroid.
					+35	9.6	68.0‖	4.8	11/ 9/46	33.0	4.8		
2 M.D.	50	F	Hypertensive	+	+35 Recurrent	13.1	67.0‖	10.0		32.0	10.0	Euthyroid in 7 wks.	In 7 wks. BMR normal and stopped fibrillating. Quinidine failed to convert auricular fibrillation when toxic.

*=I^{131}.
†=Auricular flutter.
‡=Probably still toxic and so classified.
§=Given KI two hours after I^{131} therapy.
‖=Based on urinary excretion.
¶=Just given a third dose of I^{131}.

TABLE 4—*Continued*

Patient (Case No. & initials)	Age	Sex	Type of heart disease	Auricular fibrill.	Before treatment			Radioactive iodine treatment				Response of patient	Remarks
					BMR, %	Serum PBI, γ %	48-hr. Uptake of tracer by thyroid, %	Dose (mc)		48-hr. Excretion, %	Total dose (mc)		
4	50	F	Hypertensive	+	+78	—	—	23.0*	8/ 2/46	—	23.0*	None.	Still toxic and fibrillating, but working and refuses further therapy.
H.F.					+27	—	75.0‖	12.0	10/21/47	25.0	12.0	Moderate	
					Recurrent								
7	70	F	Arteriosclerotic	0	+46	—	16.0‖	12.0		71.0	12.0	—	KI treatment until 2 mos. of I¹³¹ treatment. Reported to have been normal in 4 mos.
M.Z.													
8	61	F	Hypertensive	0	+72	—	84.0‖	10.0	5/ 2/48	13.0		Gradual	Responded in 3 mos. after 3rd dose.
M.H.					+60	—	—	7.0	1/31/49	25.0	25.0		
					+20	—	52.0	8.0	12/17/49	51.0			
9	62	F	Hypertensive	0	+74	18.6	92.0‖	10.0		6.3	10.0	—	Died 19 days after treatment at home of a myocardial infarct while hanging curtains.
J.C.													
10	78	F	Arteriosclerotic	+	+25	—	47.0‖	8.0		57.1	8.0	Euthyroid in 4 mos.	Previous KI treatment until 1 mo. of I¹³¹ treatment—still fibrillating.
G.D.													
11	76	F	Hypertensive	+	+40	—	58.0	8.0		39.2	8.0	Euthyroid in 4 mos.	KI therapy until 40 days of I¹³¹ therapy—still fibrillating.
B.N.													
12	54	F	Hypertensive	+	+38	13.4	79.0‖	10.0	6/13/47	31.0		Slight	Still toxic and fibrillating—refuses further treatment.
T.R.							69.0‖	10.0	1/28/48	32.0	20.0	Slight	
13	73	F	Arteriosclerotic & hypertensive	0	+62	—	—	25.0*	5/17/46	75.0	25.0*	Myxedema 6 mos. after 2nd dose	On KI until 2 wks. before first dose of radioactive iodine.
M.C.					+26	—	—	10.0	6/ 7/47	25.0	10.0		
15	61	F	Hypertensive	+	+44	—	75.0	10.0		6.0	10.0	Euthyroid in 3 mos.	Digitalis stopped in 3 mos. and no recurrence of heart failure. Regular rhythm.
L.P.													
16	72	F	Hypertensive	0	+50	—	78.0	7.0		—	7.0	Euthyroid in 3 mos.	Heart failu e still troublesome though euthyroid. Severely hypertensive with large hiatus hernia. Died 6 mos. after treatment of a cerebrovascular accident.
I.F.													
21	58	M	Hypertensive	+	+31	14.3	82.0‖	?		9.3	?	Euthyroid in 2 mos.	Myxedema in 4 mos. Fibrillation disappeared in 3 mos.
C.C.F.													
22	72	F	Arteriosclerotic	+	+27	—	—	8.0		20.4	8.0	Slow response over 1 yr.	Euthyroid, but still fibrillating. Digitalis needed to control failure.
M.P.													
27	85	F	Arteriosclerotic & hypertensive	0	+38	12.8	62.0	11.0	11/ 8/49	12.0	26.0	Gradual over 1 yr.	Large multinodular goiter. Euthyroid 6 mos. after 2nd dose of I¹³¹. Pulse 120, and regular on digitalis and quinidine, while toxic.
A.W.					+18	10.8	—	15.0	6/13/50	30.8			
					Nodular								
36	67	M	Arteriosclerotic	+	+40	12.0	86.0	8.0	3/ 9/50	17.6	18.0	Slight	Still toxic—heart failure developed, with a BMR of −8 per cent; taking digitalis and propylthiouracil (300 mg./d).
P.S.					+35	14.7	66.0	10.0	11/ 9/50	10.2		Slight	
								¶	5/ 7/51				
39	60	F	Arteriosclerotic & hypertensive	0	+50	20.7	82.0	10.0	6/10/50	12.9	19.0	50% better	Euthyroid 2 mos. after 2nd dose.
W.P.					+32	13.3	72.2	9.0	12/ 7/50	35.4		Good	
42	53	F	Hypertensive	0	+50	—	92.0‖	10.0	3/16/48	11.0			
M.L.					+26	—	95.0‖	6.0	12/16/48	8.7	27.0	50% better after 1st dose. Euthyroid in 2 mos. after 3rd dose	Worse, and goiter larger after KI treatment before I¹³¹ therapy.
					+14	13.5	84.0	11.0	5/ 9/50	9.8			
43	72	F	Hypertensive	0	+29	—	81.0	10.0		41.4	10.0	Euthyroid in 3 mos.	Cerebrovascular accident and a right hemiplegia 4 mos. after therapy.
M.B.													

TABLE 4—*Continued*

Patient (Case No. & initials)	Age	Sex	Type of heart disease	Auricular fibrill.	Before treatment			Radioactive iodine treatment				Response of patient	Remarks
					BMR, %	Serum PBI, γ %	48-hr. Uptake of tracer by thyroid, %	Dose (mc)		48-hr. Excretion, %	Total dose (mc)		
44 W.H.	60	M	Hypertensive	+	+20 Nodular	10.7	60.0	8.0		22.0	8.0	Euthyroid in 2 mos.	KI added after I^{131} therapy. See Fig. 8b and Fig. 9.
14 A.H.	76	M	Coronary heart disease	+	+35	9.5	85.0‖	10.0		25.0	10.0	Euthyroid	KI after I^{131} treatment, therefore difficult to assign a time response. Angina improved and auricular fibrillation ceased. Cerebrovascular accident 2 yrs. after therapy.
17 F.B.	70	F	Coronary heart disease	0	+35	10.6	62.0‖	12.0		40.0	12.0	Euthyroid in 3 mos.	Angina relieved in 3 mos. Recurred 2 yrs. after treatment. Patient died of a coronary occlusion, though euthyroid.
19 C.C.	58	M	Coronary heart disease	+	+22	17.3	44.0‖	12.0		59.0	12.0	Gradual. Euthyroid in 6 mos.	KI until 1 mo. before I^{131} treatment. Stopped fibrillating in 6 mos., when PBI normal. (See Fig. 1.) No angina when euthyroid.
20 J.S.	62	M	Coronary heart disease	0	+44	—	83.0‖	8.0		19.0	8.0	Euthyroid in 4 mos.	No angina when euthyroid.
35 G.B.	70	M	Coronary heart disease	+	+28	18.6	80.0	10.0		11.3	10.0	Euthyroid	Difficult to assign a time response. Still fibrillating.
38 W.H.	56	M	Coronary heart disease	0	+45	12.9	76.0	9.0		17.8	9.0	Gradual over 6 mos.	PBI = 7.5 in 6 mos. and angina practically gone. Angina developed while taking 300 mg. of propylthiouracil and supposedly euthyroid.
41 P.K.	67	M	Coronary heart disease	0	+48	—	72.0	10.0	5/ 3/49	4.2		Slight	Euthyroid 2 mos. after 2nd dose. See text and Fig. 2.
					− 8	11.8	61.0	10.0	4/28/50	53.1	20.0	Good	
45 J.E.	54	M	Coronary heart disease	0	+40	—	—	28.0*		20.0	28.0*	Myxedema in 4 mos.	Angina completely relieved when euthyroid.
3 M.L.	57	F	Rheumatic heart disease	+	+45	—	78.0‖	8.6		24.0	8.6	Euthyroid in 2 mos.	Stopped fibrillating when euthyroid.
18 I.S.	54	F	Rheumatic heart disease	+	+23	12.6	30.0‖	10.0		45.0	10.0	Euthyroid in 3 mos.	Taking KI until 1 mo. before I^{131} treatment. Receiving digitalis now. Fibrillating slowly. Requires mercuhydrin weekly to control heart failure.
23 C.C.	49	F	Rheumatic heart disease	+	+33 Nodular	6.9	67.0‖	10.0		33.8	10.0	Gradual. Euthyroid in year	Recurrent episodes of heart failure in spite of digitalis, when toxic. Hyperfunctioning nodule responding to I^{131}.
34 E.C.	42	F	Rheumatic heart disease	+	+50	4.7	70.0	10.0		54.4	10.0	Propylthiouracil given after I^{131}.	Previous KI therapy before I^{131} therapy.
5 B.A.	55	F	?	+	+45	—	88.0‖	14.0		16.0	21.0	Slight	Normal, with regular pulse 2 mos. after 2nd dose.
					+18		75.0‖	7.0		58.3		Good	
28 M.S.	63	F	?	+	Recurrent +55	—	99.0	10.0	2/10/50	29.0	22.0	Euthyroid 4 mos. after 2nd dose	Paroxysmal auricular fibrillation, though euthyroid
					+47	13.5	80.0	12.0	6/17/50	18.7			
32 R.W.	41	M	?	+	+55	+	92.0	10.0		22.5	10.0	Good. Normal in 4 mos.	BMR still elevated though euthyroid. Still has auricular fibrillation.

dosage of the isotope required, and the effect on the general health of the patient during the response period (Table 4). Our failures are considered. In no instance was a patient made worse by treatment.

As can be seen from Table 5, after a single dose 21 patients were rendered euthyroid in a time interval extending from one to twelve months; 17 of these 21 responded within four months; 5 became myxedematous within four months, and 4 remained toxic (Cases 9, 29 and 40).[1]

TABLE 5. THE RESPONSE TO 1, 2 AND 3 DOSES OF RADIOACTIVE IODINE

Doses (No.)	Still toxic	Euthyroid						Myx-edema	Total
		1 mo.	2 mos.	3 mos.	4 mos.	6 mos.	1 yr.		
1	4A	1	4	7B, C	5	2	2	5	30
2	3		3		1	1		1	9
3	2		1	1					4
Total	9				28*			6	43

5 died: A—(J.C., 19 days after treatment (R_x); A.L., 5 wks. after R_x; R.L., 5 yrs. after R_x.) B—(F.B., 2 yrs. after R_x.) C—(I.F., 6 mos. after R_x.)

* Four other patients responded, but we are unable to determine the exact time interval required to reach the euthyroid state.

After a second dose, 5 patients became euthyroid over a period of from two to six months, 1 became myxedematous in six months, and 3 remained toxic. Of the latter 3, one (Case 4) refuses further therapy and is busy managing her store; another (Case 12) has a normal basal metabolism but an elevated serum PBI and 48-hour tracer uptake, and is fibrillating slowly; the third (Case 36) has just been given a third dose.[2]

After a third dose of radioactive iodine, 2 patients became euthyroid in two and three months, respectively, and 2 remained toxic (Cases 24 and 46).

In all, 38 of these 47 patients became euthyroid or hypothyroid. Nine patients were still toxic after treatment; after one dose, 3 of the 4 who did not respond died while still toxic; after two doses, 2 refused further therapy and 1 has just been given a third dose[2]; after 3 doses, 1 had a slight response, and the other (Case 46), was considered as a complete failure and has recently had a subtotal thyroidectomy (Fig. 6).

No patient has been given more than three doses, and the largest single

[1] Hereafter only patients with numbers are included in the cardiac series; the others are among the 330.

[2] This patient has been considered as having had 2 doses in this report (see Table 4).

dose of radioactive iodine given has been 15 millicuries. The largest total dose given was 40.4 millicuries (Case 46). The longest interval over which one of these patients has been followed and treated without becoming euthyroid and yet responding in part and able to work has been in patient E.DeC. (Case 24), whom we have followed since August 1948.[3]

Deaths in the course of the disease have numbered 5. Patient J.C. (Case 9) had severe congestive failure while in the hospital and was markedly improved after radioactive iodine therapy. However, three weeks after returning home she disregarded our advice to rest during the average response period of two months and had a fatal coronary occlusion while hanging curtains. Patient A.L. (Case 47) lived in Western Massachusetts and was still severely thyrotoxic in spite of four partial thyroidectomies. Four weeks after radioactive iodine therapy, while ill with a respiratory infection, she travelled 150 miles to the Clinic for her regular check-up; she should have been hospitalized but she returned home and died of pneumonia and heart failure a week later in a local hospital. Patient R.L. (Case 29) had three thyroidectomies over a period of twenty-two years and had taken iodine irregularly without benefit. Agranulocytosis had developed while she was taking thiouracil. After treatment with radioactive iodine (I^{130}) in December of 1945, she was greatly improved and was able to withstand an episode of pneumonia in 1948. Despite several normal BMR's her serum PBI in 1950 was 10.5 gamma per cent and a tracer uptake was 65 per cent in forty-eight hours. Although she protested in 1950 that she was well and worked regularly, she died of causes unknown in another hospital in 1951. Patient I.F. (Case 16), a severely hypertensive 72-year-old female, responded to treatment in four months and died from a cerebrovascular accident two months later. Patient F.B. (Case 17) died two years after therapy; she had great relief from angina and became euthyroid in three months; her metabolic rates were persistently high, yet repeated PBI's were normal; she had a normal 48-hour tracer uptake four weeks before her death from coronary occlusion. Her case is discussed in detail in Cabot Case #36471 (17).

Response of the arrhythmia and congestive failure to therapy

Consideration of the general well being of the patient includes the cardiac status. Plummer (18) in 1925 stated that, although digitalis helped to alleviate congestive failure in thyrotoxic patients, it was toxic to the central nervous system and caused cerebral edema. He believed that this occurred without over-digitalization, especially in the older patients. Ler-

[3] Given a fourth dose of 11 mc. on June 15, 1951 (total, 39 mc.), and has responded.

man and Means (19) found the incidence of auricular fibrillation to be 11.5 per cent in thyrotoxic males and 6.8 per cent in thyrotoxic females. They believed that this sex difference may not have been real because there was a difference in age sampling. Hamilton (20) said that only a few patients with established auricular fibrillation would revert to a normal rhythm when euthyroid. Means (21) concluded that either paroxysmal or long continued auricular fibrillation usually disappeared with the termination of the thyrotoxicosis. Griswold (22) stated that the one phenomenon that may not revert to normal (with operation alone) in the majority of cases was auricular fibrillation.

In our experience we have seen cardiac symptoms persist in spite of therapy with the antithyroid drugs and iodide that had apparently ren-

TABLE 6. THE RESPONSE OF AURICULAR FIBRILLATION IN 27 THYROTOXIC PATIENTS TO TREATMENT WITH RADIOACTIVE IODINE. (WHEN 12 OF THESE 27 PATIENTS BECAME EUTHYROID THE RHYTHM REVERTED TO NORMAL WITHOUT THE USE OF QUINIDINE. THE 15 PATIENTS WHO STILL HAD FIBRILLATION WERE EITHER STILL TOXIC, OR HAD SEVERE ORGANIC HEART DISEASE.)

Still toxic, and still fibrillating	Euthyroid, and no fibrillation	Euthyroid, and still fibrillating
7	12	8

dered the patient euthyroid. Patient W.H. (Case 38) had angina at a time when he was supposedly euthyroid during long continued treatment with propylthiouracil. Since radioactive iodine therapy, his angina has practically disappeared. Patient P.S. (Case 36) had congestive heart failure with a BMR of −8 per cent while taking propylthiouracil and digitalis.

In our series of 27 patients with auricular fibrillation, the use of digitalis with other cardiac measures helped to alleviate the congestive failure and caused a decrease in the ventricular rate to about 100. However, it has seldom been possible to reduce the ventricular rate below 90 in these patients. It is difficult to define digitalis sensitivity or toxicity but we have repeatedly observed digitalis intoxication from attempts to force the rate down below 100 by giving more digitalis.

From Table 6 it may be seen that in 12 of 27 patients with auricular fibrillation the cardiac rhythm reverted to normal after radioactive iodine therapy in from one to six months—all without the use of quinidine. In patient R.C. (Case 30) the auricular fibrillation was converted to a regular rhythm only temporarily by quinidine before treatment with radioactive iodine. Patient J.M. (Case 37), with auricular flutter, has had no further arrhythmia since becoming euthyroid. We believe that an impor-

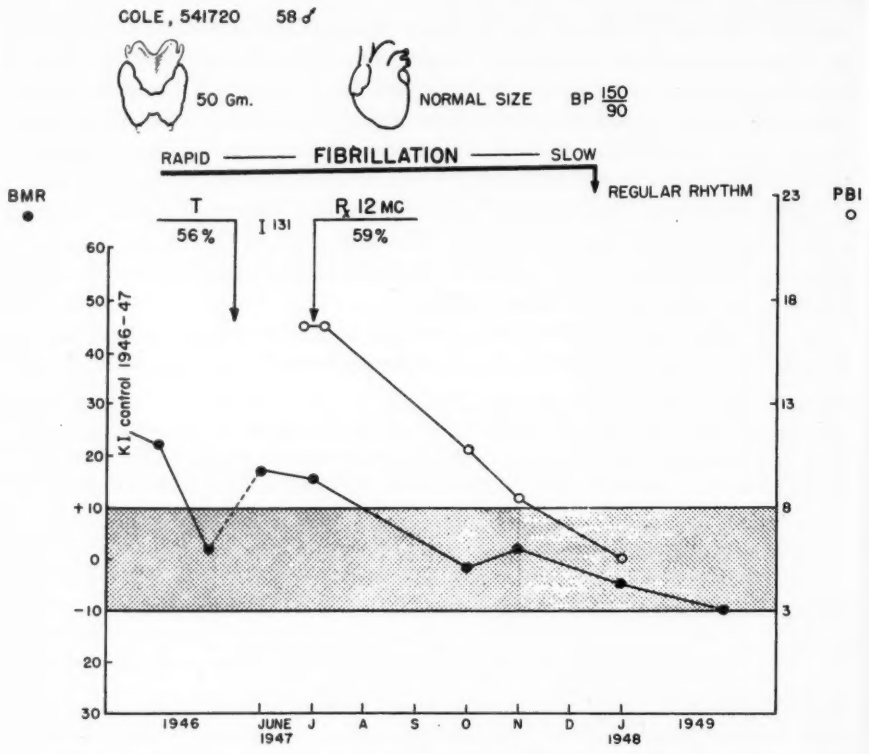


FIG. 1. The response of auricular fibrillation in a thyrotoxic patient (Case 19) to treatment with radioactive iodine only. Persistent auricular fibrillation in spite of normal BMR's, and reversion of the irregularity to a normal rhythm only when the serum PBI was normal.

tant guide to the problem of fibrillation is the serum PBI. An example of this may be seen in Figure 1. The restoration of regular rhythm occurred only when the PBI became normal. Among 7 (of the 27) patients with persistent toxicity and fibrillation, 2 (Case 4 and Case 29) had normal BMR's but elevated PBI's. Eight (of the 27) whose auricular fibrillation persisted, even when they were euthyroid, had severe organic heart disease. One patient of the 8 (Case 23) with rheumatic heart disease and thyrotoxicosis as a result of a single hyperfunctioning nodule entered our emergency ward nine times in one year with congestive failure in spite of digitalization. Since becoming euthyroid after radioactive iodine therapy she has been in the hospital once because of a respiratory tract infection and fear of heart failure. In our experience, persistent auricular fibrillation indicates either continuing hyperthyroidism or severe underlying organic

heart disease necessitating digitalis and other measures to control heart failure.

Angina pectoris

Möbius (23) in 1896 mentioned that angina pectoris was rarely present in patients with thyrotoxicosis. Haines and Kepler (24) stated that "the frequency of the association is slightly higher than is ordinarily supposed, for it is very easy to overlook angina pectoris in the presence of severe hyperthyroidism, or mild hyperthyroidism in a patient with angina pectoris."

In all 8 of our patients with angina pectoris, this disability either disappeared completely or was greatly ameliorated when the hyperthyroidism was controlled. Patient F. B. (Case 17; Cabot Case #36471) has been re-

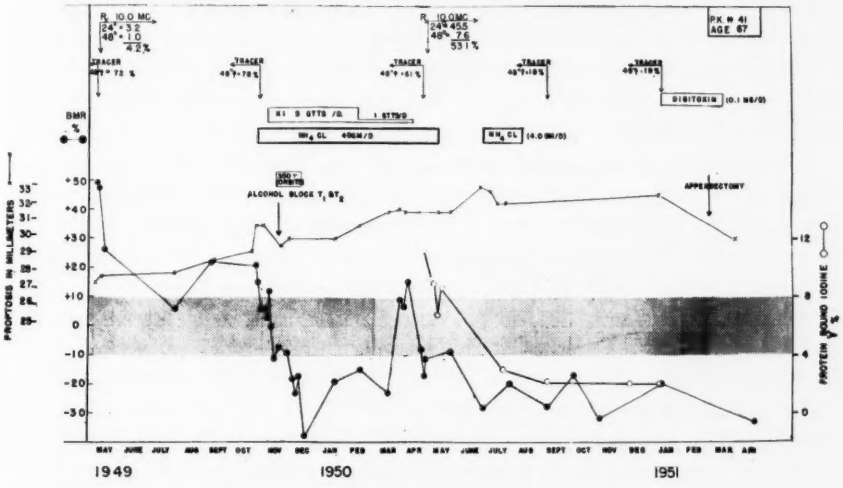


FIG. 2. The course of a 67-year-old male patient (Case 41) with angina decubitus and malignant exophthalmos, treated with radioactive iodine. See text for details.

ferred to previously. In Patient W.H. (Case 38) angina developed while he was taking propylthiouracil (300 mg. daily); he had been taking it for two years, during which time he was supposedly euthyroid. Six months after his treatment with radioactive iodine he was practically free of angina. Patient P.K. (Case 41) merits a detailed discussion. His course may be seen in Figure 2. In spite of his severe angina, the thyrotoxicosis was not overlooked. Briefly, his history is as follows:

This 67-year-old white retired male was admitted to the Massachusetts General Hospital in April of 1949 with a diagnosis of angina decubitus, of one week's duration. He claimed to be well except for mild exertional dyspnea for five months, and symptoms suggesting thyrotoxicosis for two months. Upon direct questioning, he admitted that his

eyes teared and burned and that occasionally he had diplopia, but he felt that the prominence had been present for years.

Physical examination revealed a nervous, hyperactive individual with evidence of obvious weight loss. His pulse was 108 and was regular. Blood pressure was 120/70 (190/108 during a severe attack of angina). The skin was warm, moist and velvety. Examination of his eyes revealed definite exophthalmos (27.5 mm. bilaterally by Hertel measurement), a great deal of puffiness of the lids, chemosis, engorgement of the lateral canthal vessels, ophthalmoplegia with impairment of gaze in all directions and marked loss of orbital resiliency. There was no papilledema. The thyroid was low-lying and difficult to delineate. However most observers believed that it was twice to three times normal size. The lungs were clear. There was a Grade II systolic murmur heard at the apex. There were no signs of heart failure.

The next question in this case was therapy. Because of the severe angina, it appeared that immediate control of thyrotoxicosis was imperative. However, the condition of his eyes was such that we feared it might be aggravated if his metabolism went below normal. His eye problem was so acute at this time that orbital decompression was considered, but the cardiologists advised that surgery was definitely contraindicated. He was treated with radioactive iodine. His course was interesting from several standpoints: 1) The partial response of the BMR to the first dose of radioactive iodine with definite partial alleviation of his angina and increase in his proptosis by Hertel measurement (though his eyes were not symptomatically worse). 2) The subsequent response of the BMR to potassium iodide, followed by a rise which may have been due to a decrease in the dosage to 1 drop per day. It was difficult to judge just what helped to relieve his angina at this time, for he received an alcohol block of T^1 and T^2 (by Dr. James C. White) with immediate relief lasting for five months. However, as the effect of the block seemed to wear off, his angina again increased even though his basal metabolic rate was about −20 per cent while he was taking potassium iodide. X-ray treatment to his orbits was suggested by Dr. Scowan of England and was given without apparent benefit to his eyes. When hospitalized before his second dose of radioactive iodine, his BMR was normal though the other two indices of thyroid function were elevated. Within one week after the second dose of the isotope his angina decreased in frequency and severity of attacks. An appendectomy was performed eight months later and he suffered no increase in his angina after the operation.

We should like to say a few words about his eyes, which presented such a problem. It should be noted that his proptosis had steadily increased after his first therapeutic dose of radioactive iodine. It is most interesting that in spite of the fact that he is somewhat hypothyroid at present (and no desiccated thyroid therapy has been given), his proptosis has not continued to increase and his eyes are better by symptom and sign.

Response of toxic nodular goiters

Crile *et al.* (25) and Richards *et al.* (26) from the Crile Clinic have reported on a series of cases of nodular goiters with hyperthyroidism treated with radioactive iodine. They believed that this isotope can control the hyperthyroidism in these cases but that large doses of radioactive iodine are needed. Crile believed that the decreased response in these patients might be due to: 1) "The presence of a large amount of colloid in which the I^{131} lost itself and was unable to irradiate the cells; or 2) variability in the activity of the different nodules, and as the more active nodules became controlled the less active ones took on more function." On the contrary, Gordon *et al.* (27) believe that nodular goiters with hyperthyroidism respond as well as diffuse goiters.

We have observed the response of toxic nodular goiters to radioactive iodine in 5 of our group of 47 patients. However, in only 3 patients (Cases 23, 25 and 44) was hyperfunction of the nodules definitely proved by the use of directional counting. Patients C.C. (Case 23) and W.H. (Case 44) were each thought to have a single nodule which was producing

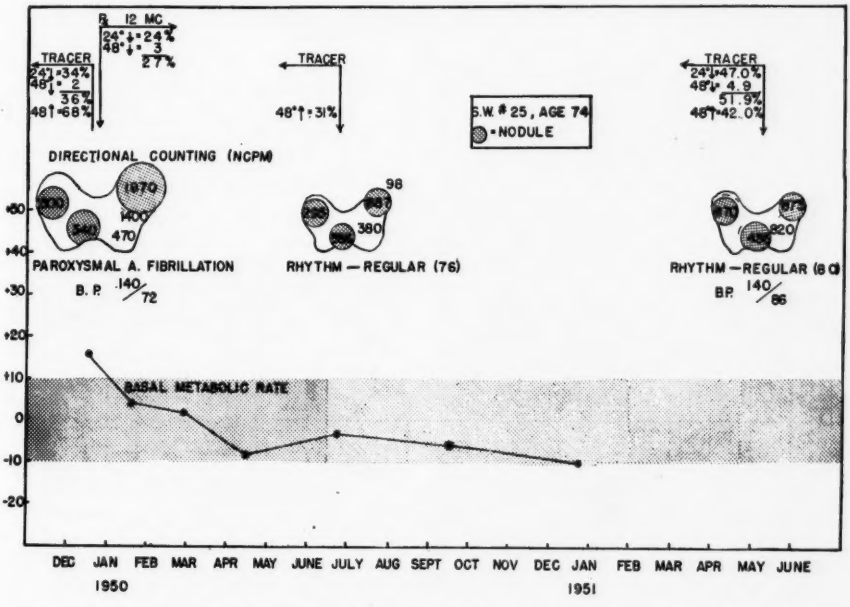
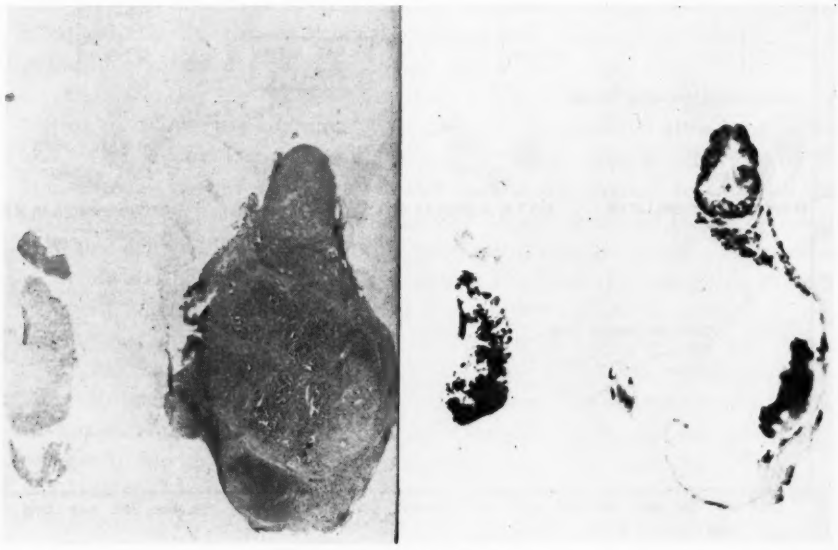


FIG. 3. The response to radioactive iodine of a multinodular goiter causing thyrotoxicosis (Case 25). A hyperfunctioning 4-cm. nodule in the left upper pole decreased to about one fourth of its original size, and the goiter as a whole decreased to some degree after therapy. There has been no recurrence of the auricular fibrillation.

the hyperthyroidism. The first patient gradually responded over a period of one year to a single dose of 10.0 millicuries of radioactive iodine. A brief description of her course has been given previously. The second patient has only recently been treated and hence no evaluation is possible at present, although the serum PBI is normal (Fig. 9). The course of Patient S.W. (Case 25) is worthy of some detail and may be seen in Figure 3. She was 74 years old and had an enlarged thyroid for at least forty years. There had been no recent enlargement. She entered with a chief complaint of palpitation and it was only after direct questioning that a few symptoms suggestive of hyperthyroidism were elicited. Physical examination revealed a large multinodular goiter (6 x) and no eye signs. During the course of observation and before therapy, auricular fibrillation was noted which reverted spontaneously to a normal rhythm. The laboratory data are as depicted. It should be noted that the large 4-cm. nodule in the left upper pole of the thyroid had at least twice the activity of any other area in the gland. The number of counts in this nodule is what we have seen in other hyperplastic nodules in patients given 500 microcuries of radioactive iodine as a tracer. She became euthyroid in two months and has been completely asymptomatic for eighteen months. Auricular fibrillation has not recurred.



Right lobe Left lobe Right lobe Left lobe

FIG. 4. Homologous sections of tissue and autoradiograph of a multinodular goiter with hyperthyroidism treated with radioactive iodine and removed surgically fourteen months later. See text for details.

The size of the gland has decreased. The "hot nodule" on the left has decreased in size 75 per cent and the activity in *this* nodule decreased by about 50 per cent. The activity in the other nodules increased slightly, suggesting that they are taking on more function. In this patient, any increased activity in the other nodules appeared to be a slow process and did not interfere with her response. We shall continue to observe these changes with great interest. Of the 2 remaining patients, A.W. (Case 27), an 85-year-old female, responded to a total of 26 millicuries (2 doses); and R. W. (Case 32) responded to a single dose of 10.0 millicuries (Table 4).

In the total series of 330 patients, we have treated few toxic nodular goiters (12 at the most) with radioactive iodine and then only because surgery was refused or contraindicated. The reason for this is obvious in Figure 4. In brief, patient R.P. presented herself with the classic symptoms of thyrotoxicosis and a multinodular goiter. One of us (Chapman) requested that a thyroid biopsy be performed before radioactive iodine

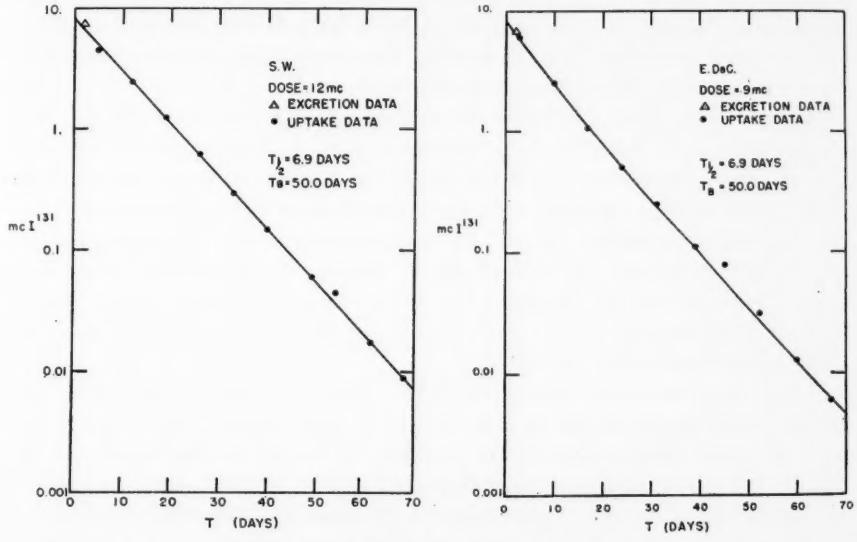


FIG. 5. The amount of retained radioactive iodine (therapy dose) in the thyroid gland as determined by weekly *in vivo* measurements over the neck. Though the biologic half-period was the same and the number of roentgens equivalent physical delivered to the thyroid (see Table 7) was approximately the same, the response was completely different.

a. (left)—Good response (Patient S.W., Case 25).

b. (right)—Little or no response (Patient E.DeC., Case 24).

T_B = biologic half-period = the time necessary for one half of the stable iodine, which is labelled with the radioactive iodine, to leave the thyroid.

$T_{1/2}$ = observed half-period = $1/T_{1/2} = 1/8 + 1/T_B$.

treatment. No evidence of malignancy was found in tissue removed from the nodule in the right lobe of the thyroid. She was treated with 8 millicuries of radioactive iodine and was estimated clinically to be much better over the course of one year. However, because of the persistent large nodule in the left lobe and mild thyrotoxicosis, the members of the Thyroid Clinic favored operation. She was given a tracer of radioactive iodine and two days later was started on saturated solution of potassium iodide, which she took for twelve days. At the end of this time a subtotal thyroidectomy was performed. The large nodule from the left lobe was found by autoradiograph study (Dr. B. Dobyns) to take up no iodine and by histologic study to be a papillary adenocarcinoma. Because of this experience we no longer treat toxic nodular goiters with radioactive iodine but advise thyroidectomy.

Failure of radioactive iodine therapy

It is very difficult to define what constitutes a failure in therapy. Should the lack of response to one dose be called a failure? Feitelberg *et al.* (28) feel that such a classification is illogical. However, from Table 5 the reader may estimate for himself the number of failures.

When one considers the crude method by which we estimate the dosage required to treat patients with thyrotoxicosis, it is no wonder that we are not always able to render these patients euthyroid with one dose. There have been various methods used for estimation of the proper dosage: 1) the estimated size of the goiter, 2) consideration of the uptake of radioactive iodine by the thyroid at 24–48 hours, 3) attempting to give a certain number of microcuries per estimated gram of tissue, and 4) estimating the dosage on the basis of attempting to deliver a specified number of roentgens equivalent physical.

All of these methods are liable to criticism, especially when we agree that the estimation of goiter size may be in error by at least 50 per cent and that the distribution of the radioactive iodine in the goiter is not always homogeneous. Fitzgerald (29) and Kelsey, Haines and Keating (6) demonstrated a patchy distribution of the retained radioactive iodine in the normal and the hyperplastic thyroid, respectively, and concluded that this may explain the variability of response to the same dose of this isotope.

Figure 5a (Case 25) and Figure 5b (Case 24) depict the amount of retained radioactive iodine in the thyroid gland after therapeutic doses, which in each case delivered approximately the same number of roentgens equivalent physical (reps.). The responses of the 2 patients were quite different (Table 7). Patient S.W. (Case 25) made a normal response in two months; patient E.DeC. (Case 24) is still toxic. Following the therapeutic dose of radioactive iodine we made weekly *in vivo* measurements

TABLE 7. THE RELATIONSHIP OF THE ESTIMATED RADIATION TO THE THYROID GLAND (R.E.P.), THE BIOLOGIC HALF-PERIOD AND THE RESPONSE OF 8 PATIENTS TREATED WITH RADIOACTIVE IODINE

Case	Age	Sex	Estimated weight of thyroid (Gm.)	Radioactive iodine				Response
				Dose (mc)	48-hr. Excretion (%)	Biologic half-period in days	Estimated radiation to the thyroid (R.E.P.)	
J.C.	34	F	100.0	10.0	8.0	33.5	11,700	Euthyroid in 2 mos.
E.DeC., Case 24	55	M	65.0	9.0	8.0	50.0	13,600	No response. See Fig. 5b.
A.D.	29	M	30.0	5.0	25.0	13.0	11,000	Moderate response—still toxic and needed 2nd dose.
A.M.	32	F	50.0	8.0	19.0	9.7	15,800	Euthyroid in 3 mos.
L.R.	54	F	40.0	7.0	11.0	14.1	21,800	Euthyroid in 2 mos.
M.S.	48	F	75.0	10.0	5.0	13.0	14,800	Euthyroid in 4 mos.
E.W.	50	F	70.0	10.0	28.0	23.8	6,900	Good response: BMR +12%, 21 mos. after I^{131} therapy.
S.W., Case 25	74	F	65.0	12.0	27.0	50.0	15,500	Euthyroid in 3 mos. See Fig. 5a.

over the thyroid of some of our patients for from eight to ten weeks. From these data[3a] the calculation of the dosage rate to the thyroid was made by means of the following formula (30):

$$R = 62 \times C \times \bar{E}$$

R = roentgens equivalent physical per day.
C = microcuries per estimated gram of thyroid tissue.
$\bar{E}$ = average energy of the beta ray = 0.205 mev.

The total dose was determined by graphic integration of the dose rate. After correcting for physical decay, the biologic half-period was determined from that portion of the curve which was exponential. Some of the curves were not exponential in the first ten days (? radiation effect). There was no consistent relationship between the number of roentgens equivalent physical delivered (6,930 to 21,800), the biologic half-period, and the response (Table 7).

Other factors have been investigated in attempting to understand the

[3a] We are indebted to Dr. G. L. Brownell for the analysis of these data.

patients who may fail to respond to treatment. W.M.L. (Case 46)[4] is a patient in whom therapy was considered to have been a complete failure. This 46-year-old woman required 400 mg. daily of propylthiouracil over a period of five months to reduce her BMR and then refused operation. She was treated with 2 doses (total 26.8 millicuries) of radioactive iodine and failed to respond. Figure 6a depicts the retained labelled iodine in the thyroid area after a tracer dose of the isotope. The biologic half-period

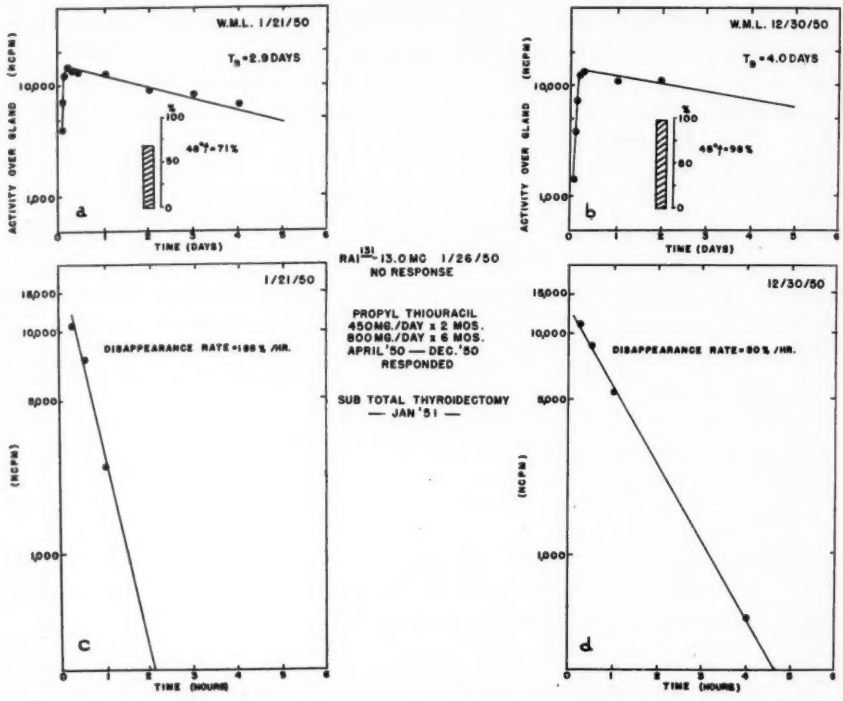


FIG. 6. The analysis of *in vivo* counting over the thyroid gland following a tracer of radioactive iodine given to Patient W.M.L. (Case 46) with Graves' disease who made no response to treatment with radioactive iodine. The activity was corrected for the physical decay. See text for details.

was very rapid at 2.9 days. This is the smallest biologic half-period that we have observed. This factor may be important in her failure to respond. We calculated the disappearance rate of iodide from the blood and found it to be 188 per cent per hour (Fig. 6c). The disappearance rate of iodide from the blood in our euthyroid patients is about 12 per cent per hour

[4] We are indebted to Dr. J. Lerman for permitting us to study this patient.

and in the usual thyrotoxic patient is about 40–72 per cent per hour. The disappearance rate was calculated with the collaboration of Dr. Gordon L. Brownell (31). The patient was then treated for the third time with 13.6 millicuries of radioactive iodine, to which she made no response over a three-month period. Propylthiouracil was then given again and in eight months her basal metabolic rate became normal. Before her subtotal thyroidectomy the studies in Figure 6 (b and d) were carried out. Propylthiouracil was stopped six days before the tracer dose was given and Figure 6b shows that the biologic half-period was still rapid at 4.0 days. The disappearance rate of iodide was now 80 per cent per hour (Fig. 6d). Histologic study of the thyroid following surgery revealed very little evidence of thyroid destruction. A detailed report on the histologic changes in the thyroid following radioactive iodine therapy in humans is in press (32).

Potential effects from the radiation

In 1948 Soley and Miller (33) reported a rise in the serum PBI from four to ten days after radioactive iodine treatment of patients with Graves' disease. They mentioned that in severe hyperthyroidism "large doses of radioactive iodine may even precipitate a serious exacerbation." Riggs (34) also reported a rise in PBI from a level of 16.2 to 23.0 gamma per cent in a patient of ours with Graves' disease after treatment with this isotope. He mentioned no exacerbation of the thyrotoxic state in this patient and indeed this did not occur. At this time Dr. J. Lerman of our Clinic suggested that the rise in the PBI following radioactive iodine therapy may have been due to the release of thyroglobulin (noncalorigenic) into the circulation.

We have obtained frequent determinations[5] of the PBI after treatment with radioactive iodine. From Figure 7 it may be seen that most of the patients treated showed a steady decline in the measurable circulating hormone. One patient (Case 31) who, before radioactive iodine treatment had auricular fibrillation and paroxysms of a rapid ventricular rate in spite of digitalis, showed a ventricular rate of 180 on the twelfth day after radiation therapy. The physician in charge believed that this disturbance of rhythm was due to a rise in the PBI and so immediately started therapy with potassium iodide. However, we secured a sample of blood on the very day of this episode before the potassium iodide was given and found no change in the level of the circulating hormone. In the 2 patients (T. McL. and M.G.) who showed a rise in the PBI, there was no exacerbation of symptoms, although 1 (M.G.) remained toxic.

Does the administration of an antithyroid drug before therapy with radio-

[5] We are indebted for these determinations to Mrs. Dorothy Pratt, Miss Eleanor Brown and Mrs. Laura Breslouf.

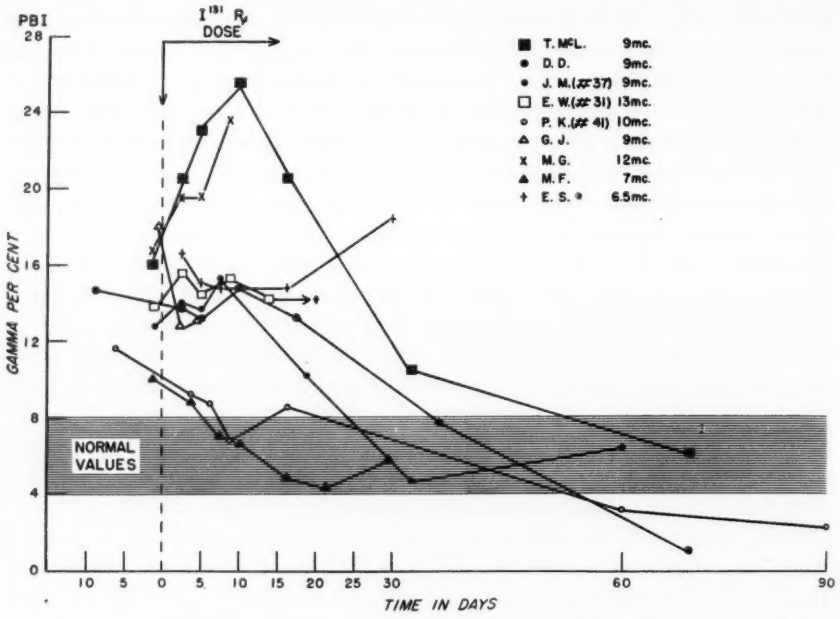


FIG. 7. The protein-bound iodine of the serum before and after therapy with radioactive iodine. A definite rise in the PBI ten days after therapy, but without aggravation of the thyrotoxic symptoms, was noted in 2 patients (T.McL. and M.G.). In Patient E.W. (Case 31), who was fibrillating, a rapid ventricular rate developed on the twelfth day of therapy; no rise in the PBI was noted. See text.

⇟ = rapid ventricular rate.

active iodine or the administration of iodide after such treatment prevent an exacerbation of the thyrotoxicosis? Williams *et al.* (35) feel that these agents do prevent a possible exacerbation. This is a very difficult question to settle but we are not sure that there has been an exacerbation of the thyrotoxic state after radioactive iodine therapy. Although our series is small, we have not been impressed with the difference in response in patients who were rendered euthyroid by pretreatment with antithyroid drugs and those who received no medication before radioactive iodine treatment. In our Clinic we have seldom used iodides after radioactive iodine therapy because our aim has been to assess the therapeutic value of radioactive iodine alone. Chapman *et al.* (36) felt that there was no difference in the response with or without added potassium iodide following radioactive iodine (I^{130}—12-hour half-life) treatment.

Stenstrom and Marvin (37) and also Freedberg and Buka (38) found that the administration of stable iodine following a therapeutic dose of

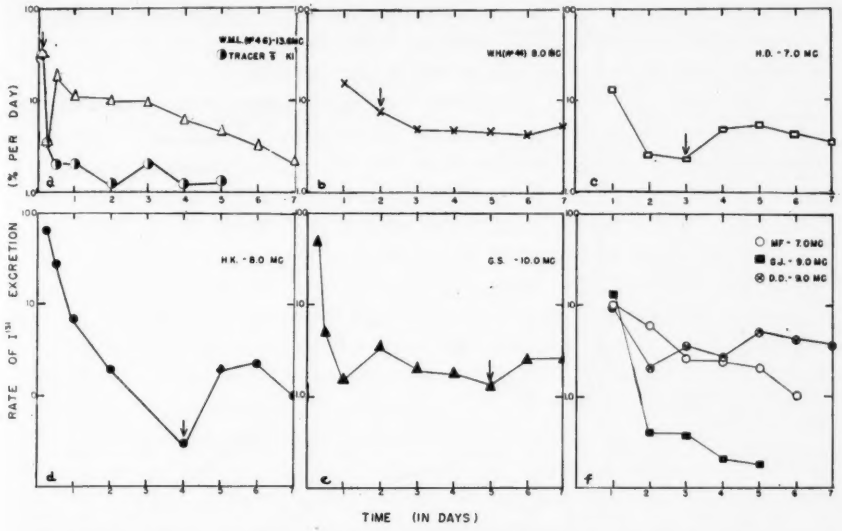


FIG. 8. The change in the urinary excretion of treatment doses of radioactive iodine after the addition of iodide, at (a) 2 hrs. (b) 48 hrs. (c) 72 hrs. (d) 96 hrs. (e) 120 hrs., and (f) with no added iodide.

a. A definite increase in the excretion of radioactive iodine. The 48-hour excretion of a tracer and a treatment dose of radioactive iodine usually agree fairly well.

b. No increase in radioactive iodine excretion.

c, d, e. Suggestive increase in radioactive iodine excretion.

f. Suggestive increase in radioactive iodine excretion in 1 "control" patient (D.D.). See text for explanation.

radioactive iodine in thyrotoxic patients caused an increase in the excretion of the isotope. Freedberg felt that this increased excretion of the radioactive iodine was much less if the stable iodine was given seventy-two hours rather than twenty-four hours after the radiation therapy. We have administered saturated solution of potassium iodide to patients, two, forty-eight, seventy-two, ninety-six, and one hundred and twenty hours after radioactive iodine treatment in order to observe any change in the serum PBI or in the urinary excretion of this isotope. No positive statement can be made as to the differences in the clinical response in these patients. In this series there was definite evidence that iodide given two hours[6] after the therapy dose caused a marked increase in the urinary excretion of radioactive iodine when we compared the excretion of the therapy dose with the previous tracer (Fig. 8a). It has been found that the 48-hour

[6] Observations in collaboration with Dr. Richard E. Goldsmith led us to believe that most of the radioactive iodine in the thyroid was protein-bound at two hours.

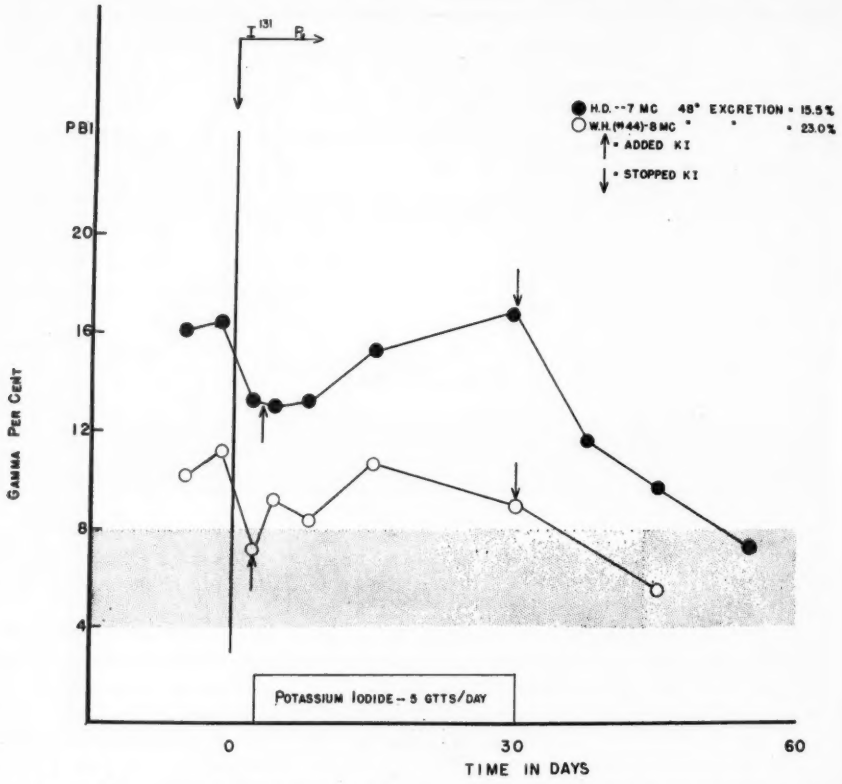


FIG. 9. The protein-bound iodine of the serum before and after therapy with radioactive iodine and added iodide (for one month). Note gradual rise in the PBI over a period of one month (especially in Patient H.D.) and noticeable drop in the PBI within one week after stopping KI.

urinary excretion values of radioactivity for tracer and therapeutic doses have been in close agreement when no iodide is given after the therapeutic dose (39). The evidence from Figure 8c, 8d,[7] and 8e[7] suggests that iodide given at seventy-two, ninety-six, and one hundred and twenty hours after therapy with radioactive iodine caused an increased urinary excretion of the isotope. The iodide given at these time intervals after the therapeutic dose may prevent the thyroid from re-utilizing the radioactive isotope. Unfortunately, we have neither coincidental *in vivo* measurements over the thyroid gland nor blood radioactive iodine studies to clarify this point. There is no rise in the excretion curve in Figure 8b, where iodide

[7] Previously reported by Chapman and Evans (14). Reproduced by courtesy of Medical Clinics of North America.

was added forty-eight hours after the therapeutic dose. One of our "control" patients (D.D., Fig. 8f) with no added iodide after 9 millicuries of radioactive iodine treatment, had a rise in the excretion of the isotope on the fifth day after therapy. This may have been due to a marked radiation effect with subsequent impairment of the thyroid's ability to re-utilize iodide; myxedema developed in this patient two months after treatment.

We have noted a rise in the serum PBI after radioactive iodine treatment and added potassium iodide in 3 cases (H.D.; W.H., #44; and W.M.L. #46). This change is depicted in Figure 9 in 2 of the patients (Cases H.D. and W.H. #44). In Patient H.D. there was a noticeable drop in the PBI within one week after the discontinuation of iodide; her PBI was normal two weeks later. In all 3 patients, while taking the iodide, there was a rise in the PBI even though the BMR and the symptoms and clinical evidence for toxicity were diminishing. This confusing paradox may be explained on the basis of studies carried out by Danowski (40) who noted a rise in the PBI, without any other evidence of thyrotoxicosis, in normal patients given iodide. We do not believe that this phenomenon is caused by the radiation effect for two reasons: first, Riggs *et al.* (41) have noted a rise in the serum PBI after administering iodide to thyrotoxic patients who were not given radioactive iodine; second, the time interval for the change in the PBI is different after the administration of radioactive iodine alone and after radioactive iodine and added iodide (Figs. 7 and 9). In all these observations, the serum was washed twice as many times as was customary to eliminate any iodide. On the basis of the lack of evidence that there is any benefit to be derived from the practice, we are now reluctant to administer iodide as a possible additive therapeutic agent following radioactive iodine treatment.

SUMMARY

Radioactive iodine is useful in the treatment of patients in whom heart disease is the chief manifestation of their hyperthyroidism. Such patients however, should follow the general medical measures for any other patient with heart disease, especially during the period of response, which is usually from two to four months. Because of the possibility of malignant disease being present in nodular goiters and despite the observed good response to radioactive iodine therapy, we now advise thyroidectomy for most toxic nodular goiters.

Radioactive iodine has been effective in causing abnormal cardiac rhythm to revert to normal without the use of quinidine. Continued auricular fibrillation after radioactive iodine therapy has indicated either:

1) Continued thyrotoxicosis—even with dissociation of the indices of thyroid function, or

2) Severe organic heart disease, which usually requires digitalis and other measures in order to control cardiac failure.

The usual response to therapy with radioactive iodine is a progressive decline in the PBI of the serum. This decrease is an important guide in treating hyperthyroid patients with heart disease. Aggravation of heart disease or of the thyrotoxic state has not been observed to follow radioactive iodine therapy.

REFERENCES

1. Hertz, S.; Roberts, A., and Evans, R. D.: Radioactive iodine as an indicator in the study of thyroid physiology. *Proc. Soc. Exper. Biol. & Med.* **38:** 510–513 (May) 1938.
2. Hamilton, J. G.: The rates of absorption of the radioactive isotopes of sodium, potassium, chlorine, bromine, and iodine in normal human subjects, *Am. J. Physiol.* **124:** 667–678 (Dec.) 1938.
3. Hertz, S., and Roberts, A.: Application of radio iodine in therapy of Graves' disease, *J. Clin. Investigation* **21:** 624 (Sept.) 1942.
4. Hamilton, J. G., and Lawrence, J. H.: Recent clinical developments in the therapeutic application of radio-phosphorus and radioiodine, *J. Clin. Investigation* **21:** 624 (Sept.) 1942.
5. Chapman, E. M., and Evans, R. D.: The treatment of hyperthyroidism with radioactive iodine, *J.A.M.A.* **131:** 86–92 (May 11) 1946.
6. Kelsey, M. P.; Haines, S. F., and Keating, F. R., Jr.: Radioiodine in the study and treatment of thyroid disease: a review, *J. Clin. Endocrinol.* **9:** 171–210 (Feb.) 1949.
7. Weinbren, M.: Radioactive iodine in the treatment of thyroid diseases: a review of the literature and preliminary report of cases treated, *South African J. Clin. Sc.* **1:** 213–289 (Sept.) 1950.
8. Parry, Caleb Hillier: Collections from the Unpublished Medical Writings of the Late Caleb Hillier Parry. London, Underwood, 1825, vol. 2, p. 111.
9. Trousseau, A.: Clinical Lectures, translated by Bazire. London, New Sydenham Society, 1868, vol. 1, p. 587.
10. Lahey, F. H., and Hamilton, B. E.: Thyrocardiacs: their diagnostic difficulties; their surgical treatment, *Surg., Gynec. & Obst.* **39:** 10, 14 (July) 1924.
11. Levine, S. A., and Sturgis, C. C.: Hyperthyroidism masked as heart disease, *Boston Med. & Surg. J.* **190:** 233–237, 1924.
12. Skanse, B.: Radioactive iodine in the diagnosis of thyroid disease, *Acta med. Scandinav.* Suppl. **235:** 1–186, 1949.
13. Dobyns, B. M.; Skanse, B., and Maloof, F.: A method for the preoperative estimation of function in thyroid tumors; its significance in diagnosis and treatment, *J. Clin. Endocrinol.* **9:** 1171–1184 (Nov.) 1949.
14. Chapman, E. M., and Evans, R. D.: Treatment of toxic goiters with radioactive iodine, *Med. Clin. North America* **33:** 1211–1233 (Sept.) 1949.
15. Hurxthal, L. M.: Heart failure and hyperthyroidism, with special reference to etiology, *Am. Heart J.* **55:** 103–108 (Oct.) 1928.
16. Maher, C. C., and Sittler, W. W.: The cardiovascular state in thyrotoxicosis, *J.A.M.A.* **106:** 1546–1557 (May 2) 1936.

17. Cabot Case #36471. Case Records of the Massachusetts General Hospital, *New England J. Med.* **243:** 828–833 (Nov. 23) 1950.
18. PLUMMER, H. S.: Use of digitalis in hyperthyroid cases, *J.A.M.A.* **84:** 1868 (June 13) 1925.
19. LERMAN, J., and MEANS, J. H.: Cardiovascular symptomatology in exophthalmic goiter, *Am. Heart J.* **8:** 55–65 (Oct.) 1932.
20. HAMILTON, B. E.: Clinical notes on hearts in hyperthyroidism, *Boston Med. & Surg. J.* **186:** 216 (Feb. 16) 1922.
21. MEANS, J. H.: The Thyroid and Its Diseases. Philadelphia, J. B. Lippincott Co., 1937.
22. GRISWOLD, D., and KEATING, JOHN H., JR.: Cardiac dysfunction in hyperthyroidism; a study of 810 cases, *Am. Heart J.* **38:** 813–822 (Dec.) 1949.
23. MÖBIUS, P. J.: Die Basedow'sche Krankheit; Nothnagel's System, Specielle Pathologie und Therapie. Wien, Alfred Hölder, 1896, vol. 22, p. 1.
24. HAINES, S. F., and KEPLER, E. J.: Angina pectoris associated with exophthalmic goiter and hyperfunctioning adenomatous goiter, *Med. Clin. North America* **13:** 1317–1324 (May) 1930.
25. CRILE, G., JR.; MCCULLAGH, E. P., and GLASSER, O.: Experience with radioactive iodine in treatment of hyperthyroidism, *Cleveland Clin. Quart.* **16:** 1 (Jan.) 1949.
26. RICHARDS, C. E.; CRILE, G., JR., and MCCULLAGH, E. P.: Radioactive iodine in the treatment of the hyperthyroidism of nodular goiter, *Tr. Am. Goiter A.* 133–139, 1950, and *J. Clin. Endocrinol.* **10:** 1077–1083 (Sept.) 1950.
27. GORDON, E. S., and ALBRIGHT, E. C.: treatment of thyrotoxicosis with radioactive iodine, *J.A.M.A.* **143:** 1129–1132 (July 29) 1950.
28. FEITELBERG, S.; KAUNITZ, P. S.; SILER, S.; SIMAN, N.; WASSERMAN, L. R., and YOHALEM, S. B.: Hyperthyroidism; treatment with radioactive iodine, *Arch. Int. Med.* **85:** 471–478 (March) 1950.
29. FITZGERALD, P. J., and FOOTE, F. W., JR.: The function of various types of thyroid carcinoma as revealed by the radioautographic demonstration of radioactive iodine (I^{131}), *J. Clin. Endocrinol.* **9:** 1153–1170 (Nov.) 1949.
30. EVANS, R. D.: Tissue dosage in radio-isotope therapy, *Am. J. Roentgenol.* **58:** 754–756 (Dec.) 1947.
31. BROWNELL, G. L.: Analysis of techniques for the determination of thyroid function with radioiodine, *J. Clin. Endocrinol.* **11:** 1095–1105 (Oct.) 1951.
32. DOBYNS, B. M.; CHAPMAN, E. M.; VICKERY, A., and MALOOF, F.: To be published.
33. SOLEY, M. H., and MILLER, E. R.: Treatment of Graves' disease with radioactive iodine, *Med. Clin. North America* **32:** 3–17 (Jan.) 1948.
34. RIGGS, D. S.: Elevation of serum protein-bound iodine after large doses of radioactive iodine, *Fed. Proc.* **7:** 251, 1948.
35. WILLIAMS, R. H.; JAFFE, HERBERT; ROGERS, W. F., JR.; TOWERY, BEVERLY, and TAGNON, RENE: Reciprocal relationships of radioiodotherapeusis and thyroid functions, (abstract) *J. Clin. Investigation* **27:** 562–563 (July) 1948.
36. CHAPMAN, E. M.; SKANSE, B. N., and EVANS, R. D.: Treatment of hyperthyroidism with radioactive iodine, *Radiology* **51:** 558–563 (Oct.) 1948.
37. STENSTROM, K. W., and MARVIN, J. F.: Urinary excretion of radioactive iodine, I^{131}, in a case of severe hyperthyroidism, *Proc. Soc. Exper. Biol. & Med.* **66:** 47–49 (Oct.) 1947.
38. FREEDBERG, A. S., and BUKA, R.: The modifying effect of inorganic iodine administered to thyrotoxic patients previously treated with RAI^{131}, (abstract) *J. Clin. Investigation* **27:** 534–535 (July) 1948.

39. CHAPMAN, E. M.: The radiation treatment of hyperthyroidism, *in* Brookhaven Conference Report, Symposium on Radioiodine. July 1948, pp. 59–63. Upton, N. Y., Associated Universities, Inc.
40. DANOWSKI, T. S., and GREENMAN, J. H.: Alterations in serum iodine fractions during the administration of potassium iodide, *Tr. Am. Goiter A.* 154–159, 1949.
41. RIGGS, D. S.; CHAPMAN, E. M., and MALOOF, F.: Unpublished data.

THE STUDY AND TREATMENT OF 119 CASES OF CARCINOMA OF THE THYROID WITH RADIOACTIVE IODINE*†

BROWN M. DOBYNS, M.D., PH.D.‡
AND FARAHE MALOOF, M.D.§

From the Thyroid Clinic and the Department of Surgery of the Massachusetts General Hospital and Harvard Medical School, Boston, Massachusetts

SINCE the time when radioactive iodine was first shown to be collected by thyroid tissue, the possibility of the collection of this radiation-producing substance by cancer of the thyroid has been under consideration (1). Investigation has subsequently been made on the uptake of radioactive iodine by various thyroidal tumors and particularly those carcinomas of the thyroid which have spread beyond the limits of complete surgical excision (1–28).

This report concerns 119 patients with carcinoma of the thyroid who have been studied with radioactive iodine at the Thyroid Clinic of the Massachusetts General Hospital for the past six years.

The purpose of this publication is to summarize some of the information that has been collected: 1) the uptake of radioactive iodine by carcinomas of the thyroid, 2) the means by which the uptake may be increased, 3) the therapeutic application in patients, and 4) the results of radiation not only in the malignant tissue but in other tissues of the body.

PLAN OF INVESTIGATION

During the course of study the following plan of investigation evolved. After a general clinical evaluation of patients with nodular goiter, tracer doses of radioactive iodine were given. Forty-eight hours later, *in vivo* directional counting (19) of the nodules in the thyroid gland was carried out and the body surveyed to detect any aberrant concentrations of radio-

Received for publication July 13, 1951.

* Read at the Annual Meeting of the American Goiter Association, Columbus, Ohio, May 26, 1951.

This article will be included in the bound volume of the "Transactions of the American Goiter Association" published by Charles C Thomas, Publisher, which will be available for sale early in 1952.

† Aided by a Grants-In-Aid from the American Cancer Society, and from the Atomic Energy Commission, and by an institutional grant to the Massachusetts General Hospital from the American Cancer Society.

‡ Present address: Dept. of Surgery, Western Reserve University, Cleveland, Ohio.

§ Public Health Research Fellow of the National Cancer Institute.

active iodine. Shortly thereafter, at the time of the operation, a pathologic diagnosis was made. If carcinoma was encountered, complete surgical excision of the disease in the neck was attempted. If this was impossible, an attempt was made to remove all normal thyroidal tissue. The completeness of this procedure was determined in many cases by applying a Geiger-Mueller counter before the surgical wound was closed. The purpose of moving all normal thyroidal tissue will be described subsequently. The uptake of radioactive iodine in the tumor and in available metastatic tissue was determined by digestion and counting, and by radioautographs.

In those patients suspected of having carcinoma of the thyroid and those subsequently found to have it, the following studies were made: diagnostic x-ray examinations of the bones and lungs; protein-bound iodine and cholesterol of serum; total protein, calcium, phosphorus and alkaline phosphatase of the blood; and functional tests of the kidney and liver. These observations were all made for the purpose of making comparisons with subsequent changes that might take place during the course of study and treatment.

At intervals following the surgical procedure, tracer doses of radioactive iodine were given: first, to confirm total excision of normal thyroidal tissue, and second, to check any changes which may have been taking place in the functional capacity of local or distant metastases. If the studies with radioactive iodine revealed that small amounts of normal thyroidal tissue remained after the operation, a dose of 25 to 50 millicuries of the isotope was given to destroy the remnant.

If the various observations indicated an increase in the uptake of radioactive iodine in the metastases following total ablation of the normal thyroidal tissue and if this increase was regarded as sufficient to be of therapeutic value, large doses of radioactive iodine were given.

As time passed, many of the patients showed little or no uptake of radioactive iodine in their metastases. Some of these patients were given various antithyroid drugs in an effort to initiate or to increase the uptake of this isotope. These results will be discussed subsequently.

Owing to a variety of circumstances, there have been variations in the method of study which has just been outlined. Some of the patients were first seen before any form of therapy had been given; however, many were referred to our clinic for study after various surgical procedures or x-ray therapy.

The observations on some of the patients do not lend themselves to a complete and critical analysis of the use of radioactive iodine in the treatment of carcinoma of the thyroid for the following reasons: 1) The malignant disease was localized and was completely removed by surgical excision. In such cases further study was only for the purpose of surveil-

lance. 2) Extensive and rapidly growing disease caused the death of the patient before the usual sequence of studies could be made. 3) Some patients have not been completely followed owing to circumstances beyond our control.

RESULTS

Classification of carcinoma of the thyroid

Carcinomas of the thyroid are difficult to classify because of the variability of the histologic pattern within the tumor. Differences in the character of various metastases within the same individual also complicate the problem. In this study an attempt has been made to classify the various carcinomas after reviewing many microscopic preparations and directing special attention to the presence of colloid formation. The importance of the formation of colloid will be pointed out in the discussion of the uptake of radioactive iodine by various types of tumors. The 119 cases that have been studied are classified in Table 1.

TABLE 1. CLASSIFICATION OF 119 CASES OF CARCINOMA OF THE THYROID

Follicular	27
Follicular and papillary	28
Papillary	22
Anaplastic and solid cellular	29
Anaplastic and papillary	5
Epidermoid	3
Lymphoma	3
Hürthle cell	2
Total	119

The initial uptake of radioactive iodine by carcinoma of the thyroid

As early as 1916 Graham (29) demonstrated that carcinomas of the thyroid contained very little iodine or thyroid hormone. We, as well as many others, have shown that the uptake of radioactive iodine in malignant thyroidal tissue is less than that of the normal gland (1, 7, 8, 9, 11, 25, 26). It has been generally found that follicular adenocarcinomas which contain colloid usually take up more radioactive iodine than other types of carcinomas of the thyroid. In contrast, the highly anaplastic carcinomas have been reported to take up none (8, 9, 11, 20). Although papillary adenocarcinoma has been considered to have little or no function (8, 9, 11) some reports have indicated that there was some uptake of radioactive iodine in such lesions (20).

A determination of the *initial* uptake of radioactive iodine was made in

TABLE 2. THE UPTAKE OF I^{131} IN CARCINOMA OF THE THYROID BEFORE AND AFTER THE REMOVAL OF THE NORMAL THYROID

Thyroidectomy	Patient: Case No. and initial	Patient: Sex	Type of Ca	Before Thyroidectomy: Digestion % dose/Gm.	Before Thyroidectomy: *In vivo*†	Before Thyroidectomy: Radioautograph†	Interval after thyroidectomy	After thyroidectomy: Digestion % dose/Gm.	After thyroidectomy: *In vivo*†	After thyroidectomy: Radioautograph†	Remarks
S**	20 D.K.	F	follicular	0 to .6*	—	—	3 mos.	.009 to 5.4*	—	—	Almost total thyroidectomy
	51 R.B.	F	follicular	—	—	slight	—	—	—	—	No recurrence 2½ yrs.
	53 G.E.	M	follicular	0	—	0	—	—	—	—	No recurrence 3 yrs.
	55 V.F.	F	follicular	—	—	0	—	—	—	—	No recurrence 2½ yrs.
	59 F.L.	F	follicular	—	0*	—	—	—	—	—	—
	110 D.P.	F	follicular	—	—	0	—	—	—	—	No recurrence 1 yr.
	3 T.I.	F	follicular	—	slight uptake*	—	21 days	—	mod. uptake*	—	Skull lesion partly excised; lesion recurred in 6 mos.; no myxedema.
	7 J.B.	M	follicular	—	slight*	—	10 days	—	—	—	28% uptake } Based on urinary excretion (see Fig. 8).
						—	42 days	—	—	—	33% uptake }
						—	12 mos.	—	fair*	—	58% uptake }
	40 M.R.	F	follicular	.002 .001 to .1*	—	0	4 mos.	.0012*	—	in one, faint metastasis	Myxedema
	61 M.M.	F	follicular	—	—	0	6 mos.	hip .19*	excellent uptake*	excellent*	TSH administration.
							7 mos.	humerus .018*	excellent uptake*	—	I^{131} therapy.
							8 mos.	humerus .03*	excellent uptake*	—	Thiouracil therapy
							20 mos.	humerus .0015*	excellent uptake*	spotty*	See Fig. 5 & Fig. 9
							32 mos.	—	excellent uptake*	spotty*	See Fig. 7
T	68 L.A.	M	follicular	.003 to .011	mod.*	0	14 days	—	excellent*	—	Subsequent I^{131} therapy.
							15 mos.	—	excellent*	mod.*	See Fig. 2
	72 C.D.	F	follicular	0.4‡	—	0	8 mos.	—	mod. uptake*	—	Myxedema after 127 mc
	79 M.S.	F	follicular	.04 .003*	0*	0*	10 days	—	excellent uptake*	—	Subsequently treated with I^{131} (see Fig. 10).
	82 B.W.	F	follicular	0 0*	0 —	0 —	7 mos. 10 mos.	0* —	— —	0* 0*	Myxedema.
	103 E.N.	F	follicular	—	0*	0	5 mos.	—	0*	—	Myxedema
	104 J.N.	F	follicular	0	0	0	2 days	—	slight uptake*	—	Lesion doubled size in 1 mo.; previous growth slow. Myxedema at 3 mos.
							1 mo.	—	slight uptake*	—	
							3 mos.	—	mod.*	mod.*	
	116 L.V.	F	follicular	.008 0 to .01*	0*	—	2 days 3 mos. 4 mos.	— — —	slight* slight* slight*	— slight* slight*	Mild myxedema; I^{131} therapy.
	118 A.W.	F	follicular	—	slight	very faint uptake*	1 mo.	slight*	mod.*	—	Skull lesion subsequently excised.
S	13 W.B.	F	papillary + follicular	.68* .02*	—	—	—	—	—	—	No follow-up.
	18 S.H.	M	papillary + follicular	.024 .0114*	—	—	—	—	—	—	No recurrence 4 yrs.
	26 I.C.	F	papillary + follicular	.015 to .025*	—	0*	—	—	—	—	No recurrence 1 yr.
	37 R.N.	F	papillary + follicular	—	—	0	—	—	—	—	No recurrence 3 yrs.

† The terms "slight," "moderate" and "excellent" in these columns represent an uptake ranging from that which is just detectable to that of therapeutic value.
* Determination on metastasis.
** S = Subtotal, T = Total } Thyroidectomy
‡ Sample may have been contaminated with normal thyroidal tissue, particularly when the primary lesion was invasive in the normal gland.

TABLE 2—*Continued*

† = Total
‡ Sample may have been contaminated with normal thyroidal tissue, particularly when the primary lesion was invasive in the normal gland.

TABLE 2—*Continued*

Thyroidectomy	Patient: Case No. and initial	Patient: Sex	Type of Ca	Before Thyroidectomy: Digestion % dose/Gm.	Before Thyroidectomy: *In vivo*†	Before Thyroidectomy: Radioautograph†	Interval after thyroidectomy	After Thyroidectomy: Digestion % dose/Gm.	After Thyroidectomy: *In vivo*†	After Thyroidectomy: Radioautograph†	Remarks
	44 C.S.	F	papillary + follicular	— to .03*	—	0*	—	—	—	—	—
	45 E.T.	M	papillary + follicular	0	—	0	—	—	—	—	Died of intercurrent disease.
S	56 E.G.	F	papillary + follicular	—	—	mod. uptake	—	—	—	—	No follow-up.
	105 L.N.	M	papillary + follicular	—	—	0	—	—	—	—	No recurrence 6 mos.
	109 R.P.	F	papillary + follicular	—	—	0	—	—	—	—	No recurrence 1 yr.
	6 H.G.	M	papillary + follicular	.0138 .0081*	—	—	2 mos.	—	slight	—	Myxedema. BMR rose spontaneously. No follow-up.
	39 C.P.	F	papillary + follicular	.076 .003 to .007*	—	0*	—	—	—	—	No follow-up.
	48 D.Y.	M	papillary + follicular	0*	—	0 0*	2 yrs.	—	slight*	slight*	I^{131} therapy; died; no postmortem exam.
	50 E.B.	F	papillary + follicular	0 to .015*	—	slight, over follicular areas	—	—	—	—	No recurrence 2 yrs. Myxedema.
	52 R.C.	F	papillary + follicular	—	—	0 0*	4½ mos.	0.46*	—	mod. uptake*	A papillary lesion in which follicles developed.
	58 R.K.	F	papillary + follicular	—	—	faint uptake	—	—	—	—	Most of thyroid previously removed. Myxedema.
	65 L.R.	M	papillary + follicular	—	—	0 0*	6 wks.	—	no uptake*	—	Very extensive metastases.
T	71 M.D.L.	F	papillary + follicular	.005 to 0.01*	—	0*	—	—	—	—	No recurrence 1½ yrs. Myxedema.
	74 G.H.	M	papillary + follicular	—	—	0*	—	—	—	—	No follow-up.
	80 J.S.	M	papillary + follicular	—	—	0	—	—	—	—	No recurrence 2 yrs. Myxedema.
	85 C.A.	M	papillary + follicular	0*	—	0 0*	2 mos.	mod. uptake	definite, but slight*	spotty uptake*	Died in 6 mos. Myxedema.
	98 E.L.	M	papillary + follicular	—	—	0	6 wks.	—	mod. uptake*	—	Acquired uptake during I^{131} therapy.
	99 J.M.	M	papillary + follicular	—	—	faint uptake	—	—	—	—	Myxedema. No recurrence 1 yr.
	112 M.S.	F	papillary + follicular	0	—	0	—	—	—	—	Myxedema; no recurrence 1 yr.
	119 G.T.	M	papillary + follicular	0	—	slight uptake*	8 mos.	—	—	—	Urinary excretion suggested uptake. Lymph node biopsy showed no carcinoma.
	1 A.E.	M	papillary	—	—	—	—	—	0*	—	Recurrence in 12 yrs.
	12 B.S.	M	papillary	.2* .04*	—	—	—	—	—	—	—
	17 A.F.	F	papillary	0 to .014*	—	—	—	—	—	—	—
S	38 E.P.	F	papillary	—	—	0	—	—	—	—	—
	43 A.S.	F	papillary	0*	—	0*	5 wks.	.001 to .01*	—	0*	See Case 43 A.S. in total thyroidectomy group.
	97 H.L.	M	papillary	0*	0*	—	—	—	—	—	—
	113 N.S.	F	papillary	—	—	0	—	—	—	—	—
	10 E.L.	F	papillary	0 0*	—	—	—	—	—	—	—
T	34 C.L.	F	papillary	0	—	0	—	—	—	—	Myxedema.
	41 F.S.	F	papillary	0*	0*	—	3 mos.	—	0*	—	Myxedema. Uptake increased by TSH.
	43 A.S.	F	papillary	.001 to .01*	—	0*	6 wks.	.003 to .02*	—	0*	—

TABLE 2—*Continued*

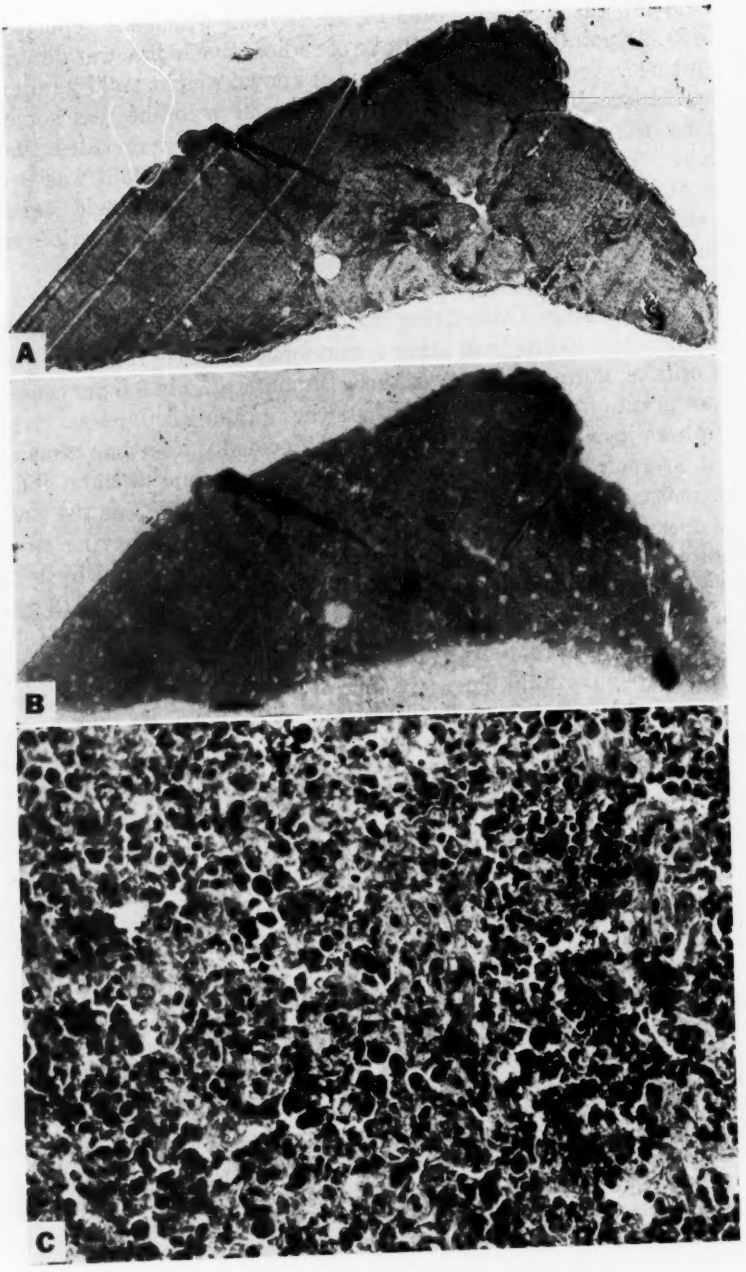
Thyroidectomy	Patient: Case No. and initial	Patient: Sex	Type of Ca	Before Thyroidectomy: Digestion % dose/Gm.	Before Thyroidectomy: *In vivo*†	Before Thyroidectomy: Radioautograph†	Interval after thyroidectomy	After Thyroidectomy: Digestion % dose/Gm.	After Thyroidectomy: *In vivo*†	After Thyroidectomy: Radioautograph†	Remarks
T	81 M.S.	F	papillary	0*	—	0*	—	—	—	—	Myxedema.
T	89 M.D.	F	papillary	0*	—	0*	6 wks.	0*	0*	—	Myxedema.
T	94 B.H.	M	papillary	0 to .01*	—	0	—	—	—	—	Myxedema.
S	60 A.L.	F	papillary + anaplastic	0	—	0* —	—	—	—	—	Died 2 days after operation.
T	11 J.S.	M	papillary + anaplastic	0 0*	—	—	—	—	—	—	No recurrence in 3 yrs.
T	77 E.N.	M	papillary + anaplastic	—	—	0*	5 mos.	0*	—	—	Myxedema. No recurrence 6 mos. after 2nd operation.
T	100 J.M.	F	papillary + anaplastic	—	—	0	—	—	—	—	Died in 2 mos.
S	25 H.B.	F	anaplastic	0*	—	0*	—	—	—	—	Died in 1 yr.
S	28 A.C.	M	anaplastic	.001 to .006*	—	mod. uptake*	—	—	—	—	Most of all normal thyroid destroyed by disease. Died in 3 mos.
S	46 L.T.	F	anaplastic	—	—	—	—	—	—	slight uptake*	Almost total thyroidectomy.
S	49 M.B.	F	anaplastic	0*	—	0*	—	—	—	—	Died in 3 mos.
S	62 E.P.	M	anaplastic	—	—	0	—	—	—	—	No recurrence in 3 yrs.
S	66 W.S.	M	anaplastic	slight uptake	—	faint uptake	—	—	—	—	Died in 2 mos.
S	75 O.L.	M	anaplastic	0	—	0	—	—	—	—	Died in 3 wks.
S	78 M.N.	F	anaplastic	—	—	0	—	—	—	—	Died.
S	83 L.Z.	F	anaplastic	0	—	0	—	—	—	—	Not followed.
S	88 B.D.	F	anaplastic	—	—	spotty faint uptake	—	—	—	—	Not followed.
S	93 H.G.	M	anaplastic	0*	—	—	1 mo.	0*	—	0 0*	I^{121} thyroidectomy. Died in 1 mo.
T	95 H.K.	M	anaplastic	0	—	0	—	—	—	—	Died in 1 mo.
T	117 O.W.	M	anaplastic	.025‡	—	0	—	—	—	—	Died in 3 mos.
T	29 M.C.	F	anaplastic	0	—	—	6 mos.	—	0	—	Myxedema. Died in 10 mos.
T	33 A.K.	M	anaplastic	—	—	—	7 wks.	—	—	mod. uptake*	Myxedema. 1 node contained carcinoma.
T							1½ yrs.	—	—	0*	Myxedema corrected. 1 node contained carcinoma.
T	35 T.L.	M	anaplastic	.0015	—	0	—	—	—	—	Died in 16 mos.
T	47 G.W.	F	anaplastic	0 to .008	—	0	—	—	—	—	Died.
T	67 L.T.	F	anaplastic	—	—	0	—	—	—	—	Died in 2 mos.
T	70 M.C.	F	anaplastic	—	—	0	4 wks.	0*	slight uptake in suspected metastases*	—	Died in 6 wks.
T	73 L.D.	F	anaplastic	.01 to .87*	—	0	3 mos.	—	—	—	Myxedema. Died in 6 mos.
T	101 E.M.	F	anaplastic	—	—	faint uptake	—	—	—	—	Uptake in one small area with colloid formation. Died in 2 mos.
S	114 J.S.	F	anaplastic	—	—	—	3 mos.	0	—	—	Myxedema.
S	14 J.B.	M	epidermoid	.003 .003*	—	—	—	—	—	—	Died.
S	15 I.C.	F	epidermoid	.001 .002*	—	—	—	—	—	—	Died.
S	108 S.P.	M	epidermoid	—	—	0	—	—	—	—	Died.
T	24 M.A.	F	Hürthle cell	0	—	—	—	—	—	—	No recurrence 4 yrs.
T	90 G.D.	F	Hürthle cell	.01*	—	0*	3 mos.	—	0*	—	Myxedema. No uptake in biopsy specimen after 5 mos. of thiouracil.
T	27 R.C.	F	lymphoma	0	—	0	—	—	—	—	—
T	69 S.B.	F	lymphoma	—	—	0	2½ mos.	—	0*	—	Myxedema; x-ray therapy; living 2½ yrs.
S	87 A.C.	F	lymphoma	—	—	0	4 mos.	—	0*	—	Myxedema. No recurrence 6 mos.

83 of the 119 cases of carcinoma of the thyroid which are reported here (Table 2). As pointed out, the uptake of radioactive iodine was determined by counting a digested sample of tissue of known weight and by radioautographs. Although a slight uptake of radioactive iodine was sometimes found by the former method, radioautographs often revealed that the radioactivity was localized in small areas in which colloid was present. These observations illustrate the importance of histologic variations when classifying these tumors and the lack of precise information yielded by the determinations of the radioactivity contained in a mass of tissue which has been digested.

It will be seen from Table 2 that the follicular adenocarcinomas collected more radioactive iodine than other carcinomas of the thyroid. The greatest *initial* uptake, however, in any of these tumors was only 0.6 per cent of the dose per gram of tissue; in most others the uptake was far less. Even the best uptake in any of these tumors was decidedly less than that of the normal thyroid. Among the other types of carcinomas without follicular components, the highest reliable assay that was found was .04 per cent of the dose per gram of tissue. Those tumors in Table 2 having an *initial* detectable uptake of radioactive iodine may be summarized by types as follows: follicular, 10 positive out of 18; follicular and papillary, 12 positive out of 24; papillary, 4 positive out of 13; anaplastic and solid cellular, 8 positive out of 19 (footnote ‡, Table 2); anaplastic and papillary, 0 positive out of 4; epidermoid, 2 positive out of 3 (footnote ‡, Table 2); lymphoma, 0 positive out of 3; and Hürthle cell, 1 positive out of 2. It should be noted, however, that in many of those listed as positive the uptake was barely detectable by only one of several methods. Because of the minute amount of radioactivity in many of these tumors and certain inadequacies in experimental technique,[1] confirmation among the methods was not always obtained. For details the reader is referred to Table 2. It was felt that although there was an initial uptake in some of these tumors, it was not sufficient to be of therapeutic value.[2]

[1] From the radioautographs that were made there is an indication that during the process of digesting samples of the primary tumor, minute fragments of normal thyroidal tissue may have been included in the tissue mass which was digested. The method may thus give a falsely elevated uptake of radioactive iodine in tumors such as the pure papillary lesions which by radioautograph have rather uniformly shown no uptake of radioactive iodine.

[2] Seidlin, Marinelli and Oshry (6) have reported a single well-documented case in which there was thyrotoxicosis and an excessive uptake of radioactive iodine in metastases. This patient had had what was thought to be almost total thyroidectomy several years before these observations were made. Several other cases have been reported. Kaae and Peterson (27) and Weinberg *et al.* (28) reported cases of carcinoma of the thyroid with hyperthyroidism. In these cases the evidence is not clear that there was an


A
B
C

Although the colloid-forming tumors took up the most radioactive iodine, there was no strict correlation between the histologic patterns of the tumors and the uptake of the isotope. Some follicular adenocarcinomas with colloid did not take up any radioactive iodine. On the other hand, a slight uptake was found in metastases from 1 anaplastic and 4 pure papillary carcinomas (Table 2). Some, but not all, of these were confirmed by radioautograph. A positive radioautographic finding in a cervical lymph node containing a highly anaplastic carcinoma (Case 28, AC ♂) is illustrated in Figure 1, A and B. The normal thyroid appeared to have been completely replaced by diseased tissue.

The effect of total thyroidectomy on the function of metastases

Means (30) in 1937 suggested that so-called benign metastases with structure similar to normal thyroid might produce sufficient thyroid hormone to prevent myxedema after the normal thyroid had been removed. Indeed, von Eiselsberg (31) in 1894 described the development of myxedema in a patient following the removal of the thyroid for malignant disease; but the myxedema disappeared coincident with the development of a metastatic lesion in the manubrium two years later. The metastasis, which was a colloid-forming adenocarcinoma, was removed and the symptoms of myxedema returned. Apparently this is the first record of the development of function in a metastasis following thyroidectomy.

In March 1945, a patient (Case 3 TI ♀) with carcinoma of the thyroid and a mildly functioning metastasis in the skull was admitted to the Massachusetts General Hospital under the care of Dr. Earle M. Chapman. At the suggestion of Dr. Rulon W. Rawson a total thyroidectomy was performed by Dr. Robert Linton to see if the uptake of radioactive iodine would increase in the metastasis. Subsequently there was an increase in the uptake in this metastatic lesion. As a result of the observations on this patient, the principle of total thyroidectomy has been applied over the past six years.

excessive uptake of radioactive iodine in the malignant tissue. A patient (93 HG ♂) in this series had a basal metabolism of +55 per cent and clinical evidence of hyperthyroidism but at the time of postmortem examination there was no uptake of radioactive iodine in any of numerous metastases.

FIG. 1. An example of a highly anaplastic metastasis (lymph node) which took up sufficient radioactive iodine to produce positive findings in a radioautograph. A = Histologic section of a metastatic lesion in the cervical area (×8). B = Radioautograph produced by radioactive iodine in A. C = High magnification (×240) of the section in A, showing a highly anaplastic tumor without colloid formation. It may be that the extensive replacement of the normal thyroid tissue by the tumor produced an effect similar to thyroidectomy.

In theory there are two reasons for eliminating the normal thyroidal function in the presence of metastases. First, since the normal thyroid picks up radioactive iodine more readily than does malignant tissue, its removal eliminates this competition and the malignant tissue has the sole opportunity to collect the isotope. Second, because an insufficiency of thyroid hormone occurs following total thyroidectomy, the metastatic tissue may develop a capacity to produce thyroid hormone. If the metastatic tissue makes thyroid hormone, it must collect and store iodine. If the tumor then collects sufficient radioactive iodine, cellular damage within the tumor will result from the radiation.

Several groups of investigators have reported the use of this principle in the treatment of carcinoma of the thyroid (8, 10, 12, 14, 22, 24). The present report[3] concerns 60 patients who have been subjected to total thyroidectomy. It will be seen in Table 3 that in 10 of these patients the disease was completely removed by surgical excision so that there was no further opportunity to study the uptake of radioactive iodine in the

TABLE 3. THYROID STRUCTURE AND METASTASES IN PATIENTS SUBJECTED TO TOTAL THYROIDECTOMY

		Follicular*	Papillary*	Papillary & follicular	Undifferentiated	Papillary & undifferentiated	Epidermoid	Lymphoma	Hürthle cell	Total
Disease confined to cervical area	Disease completely excised	1	3	5	0	1	0	0	0	10
	Disease incompletely excised	4	4	7	3	2	0	2	0	22
Distant metastases		11	5	4	7	0	0	0	1	28
Total		*16*	*12*	*16*	*10*	*3*	*0*	*2*	*1*	*60*

* Essentially pure histologic pattern.

malignant tissue. There were distant metastases in 28 patients. In 22 other patients the disease, although confined to the cervical region, could not be completely excised. Special interest was directed toward those patients who had lesions which formed acini, either follicular or papillary and follicular (Table 3). Twenty-six of these patients had local or distant metastases after the total thyroidectomy had been accomplished.

In Table 2 the patients who had total thyroidectomy have been classified according to histologic types. As pointed out, the uptake of a tracer dose of radioactive iodine has been estimated by one or several of the following methods: 1) *in vivo* counting over a metastatic site, 2) radioautographs,

[3] Some of these cases were previously reported (8, 14, 22) from the Thyroid Clinic of the Massachusetts General Hospital.

and 3) digestion of the tissue removed. These observations have been repeated over a period of from several days to years after total thyroidectomy. The ability of follicular adenocarcinomas to collect radioactive iodine has been either acquired or increased in some instances. This has been observed in metastases from 11 (2 initiated, 9 increased) of 13 follicular, and from 4 (all initiated) of 5 mixed follicular and papillary carcinomas. In many of these the increase was sufficient to be of striking therapeutic value. Although myxedema developed in the patients with pure papillary adenocarcinoma in only 1 of 3 of this type was an uptake of radioactive iodine initiated and in none was it increased. In 3 patients with anaplastic

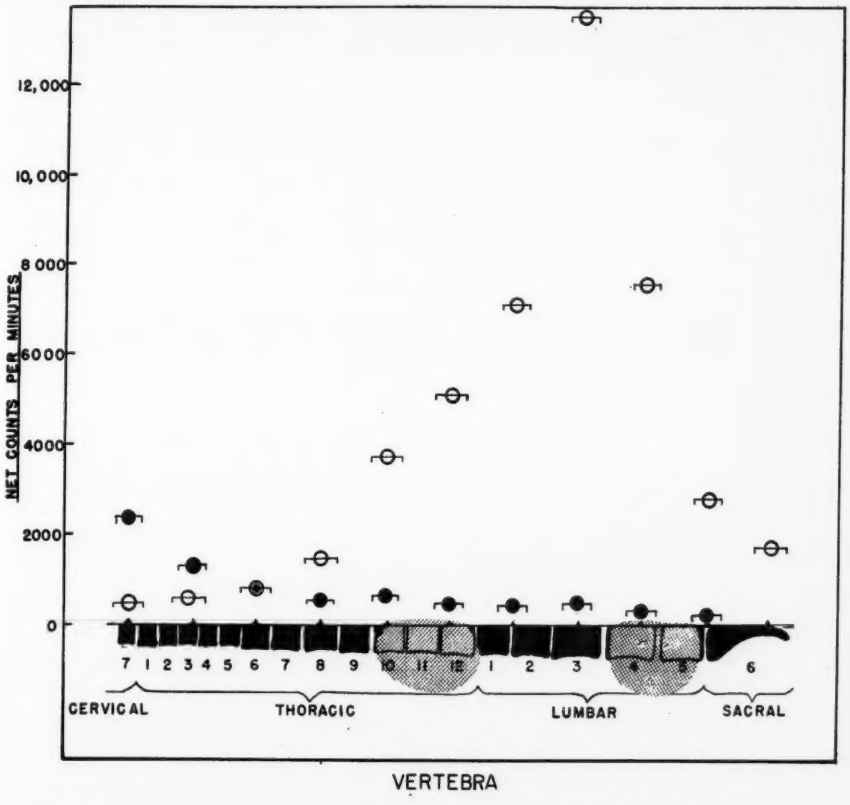


FIG. 2. The change in the uptake of radioactive iodine in metastatic follicular adenocarcinoma of the thyroid following total thyroidectomy. Identical tracer doses of the isotope were given prior to and fourteen days following total thyroidectomy. *In vivo* counting over the vertebral column revealed an increased concentration of the isotope as the area of the thyroid was approached preceding the operation (shaded circles) and in the area of the metastases after the operation (open circles).

TABLE 4. THE EFFECT OF TOTAL THYROIDECTOMY ON THE BMR, SERUM PBI AND EXCRETION OF I^{131}

Patient		Type of Ca	Before thyroidectomy			Interval after thyroidectomy	After thyroidectomy			Remarks
Case No. and initial	Sex		BMR, %	PBI, γ %	I^{131} excretion in 72 hours (% of dose)		BMR, %	PBI, γ %	I^{131} excretion in 72 hours (% of dose)	
3 T.I.**	F	follicular	−11	—	—	2 wks.	+10	—	—	I^{131} uptake in lesion in skull increased.
						10 mos.	—	—	65.0	
						28 mos.	−17	3.2	92.0	Receiving 1 grain of thyroid daily.
7 J.B.**	M	follicular	+ 7	—	66.4*	1 wk.	−14	—	72.2*	No myxedema. Increased uptake of I^{131} over metastatic lesion in spine.
						5 wks.	−20	4.4	67.5*	
						56 wks.	−10	3.0	42.6	
40 M.R.	F	follicular	+20	4.1	55.9	3 wks.	—	2.8	80.9	Thyroidectomy not quite complete. Slight uptake of I^{131} subsequently.
54 A.E.**	M	follicular	—	—	63.4 44.4					No uptake of I^{131} in metastases.
					39.8	6 days	—	—	99.0	No follow-up.
61 M.M.	F	follicular	+ 8	—	59.0*	6 mos.	+10	4.9	20.6	I^{131} uptake in lesions in humerus and ilium increased.
68 L.A.	M	follicular	−27	—	44.9	14 days	—	6.4	35.6	I^{131} uptake in lesion in spine increased.
72 C.D.**	F	follicular	− 1	—	32.5	7 days	—		63.0	Disease confined to cervical region. Possibility of small remnant of normal thyroid; 35 mc of I^{131} given at 5 wks. to destroy remnant.
						12 wks.	—	1.5	71.8	
79 M.S.	F	follicular	—	4.9	60.0*	1 wk.	—	—	53.0*	I^{131} uptake, previously absent occurred in ilium.
						5 wks.	− 5	4.9	58.0*	
82 B.W.**	F	follicular	−11	—	89.9	64 days	−21	—	90.3	Myxedema.
						7 mos.	−31	—	97.0	No I^{131} in biopsy specimen of tumor.
84 M.A.**	F	follicular	− 7	—	54.0	51 days	—	—	98.0	No known distant metastases.
96 C.K.**	M	follicular	—	—	—	34 days	—	—	102.0	Myxedema. Respiratory obstruction.
103 E.N.	F	follicular	—	—	71.0	3 days	—	—	88.0	
						6 wks.	−20	—	93.0†	No uptake of I^{131} in metastases.
104 J.N.	F	follicular	− 6	4.1	65.0	4 days	—	—	85.0	No uptake of I^{131} initially in skull lesion.
						30 days	myxedema	0.2	65.0	Slight uptake 2 days postop.
107 M.P.**	F	follicular	—	—	—	2 yrs.	—	—	96.1	Total thyroidectomy—Lahey Clinic. No subsequent increase in uptake of I^{131}. Tracheotomy.
116 L.V.**	F	follicular	− 1	—	82.7	4 days	—	—	85.0	I^{131} uptake, previously absent, occurred in sacral lesion (slight).
118 A.W.**	F	follicular	− 8	—	51.8*	3 days	—	—	74.3	I^{131} uptake in lesion in skull.
						20 days	—	—	63.5	Subsequently excised.
6 H.G.	M	follicular +papillary	− 1	—	71.1	3 mos.	myxedema	3.8	63.9	Definite metastasis not proven.
~~48 D.Y.~~	M	follicular +papillary	—	—	61.0	56 days	—	—	69.0*	I^{131} uptake in cervical nodes subsequently demonstrated.
						7 mos.	−30	1.6	87.0	
50 E.B.	F	follicular +papillary	—	—	51.5	29 days	−22	—	—	Treated with desiccated thyroid. No evidence of recurrence.
52 R.C.	F	follicular +papillary	−19	—	70.2	5 wks.	−25	—	42.6*	Not quite total; 10 mc of I^{131} given at 8 weeks.
						16 wks.	−27	—	46.9*	Metastases subsequently took up I^{131}.
58 R.K.**	F	follicular +papillary	−16	—	—	6 days	—	—	98.6	Myxedema; short follow-up.
65 L.R.	M	follicular +papillary	−29	—	—	8 wks.	myxedema	—	no uptake	No follow-up.
71 M.D.L.**	F	follicular +papillary	—	—	69.0	10 days	—	—	74.0*	No follow-up.
						22 days	—	—	83.0	

** Previous thyroid operation.
* Only 48-hour urinary excretion.
† Calculated on the basis of *in vivo* uptake.

TABLE 4—*Continued*

Patient		Type of Ca	Before thyroidectomy			Interval after thyroidectomy	After thyroidectomy			Remarks
Case No. and initial	Sex		BMR, %	PBI, γ %	I^{131} excretion in 72 hours (% of dose)		BMR, %	PBI, γ %	I^{131} excretion in 72 hours (% of dose)	
80 J.S.	M	follicular +papillary	−16	—	68.0	4 days	—	—	95.0	No evidence of disease remained.
85 C.A.	M	follicular +papillary	−22	6.2	—	2 wks.	—	4.6	73.9	Not quite total; I^{131} used subsequently.
						21 wks.	—	1.2	53.9	
98 E.L.**	M	follicular +papillary	− 7	5.8	82.0	5 wks.	—	—	88.6	I^{131} uptake subsequently in cervical metastases.
						16 wks.	−20	0.5	91.4	
105 J.M.	M	follicular +papillary	−17	—	—	7 days	—	—	81.1	Definite metastases not proven.
						2 mos.	−23	1.1	85.6	
						3 mos.	−38	0.7	82.6	Myxedema in 3 months.
112 M.S.**	F	follicular +papillary	—	—	—	10 days	—	—	99.0	
						7 mos.	myxedema	—	93.0	Re-explored; no disease found.
115 A.T.	F	follicular +papillary	− 7	—	—	10 days	−35	0.2	no uptake	Myxedema. No evidence of recurrence.
119 G.T.	M	follicular +papillary	—	—	30.9	8 mos.	− 8	—	34.2	Received thyroid medication shortly after operation; subsequent biopsy specimen contained no carcinoma.
10 E.L.	F	papillary	−10	4.0	58.0	10 wks.	−40	1.6	91.9	Not quite total. I^{131} therapy.
22 R.W.	F	papillary	−15	—	—	7 days	−24	—	95.0	
						1 yr.	—	—	98.8	
34 C.L.	F	papillary	—	—	—	26 days	—	1.3	97.9	Myxedema.
41 F.S.	F	papillary	+11	—	61.5	3 days	—	—	95.8	No uptake of I^{131} in metastases.
						19 days	—	—	99.6	Subsequently uptake, after TSH.
81 M.S.	F	papillary	—	—	55.0	6 days	—	—	95.6†	Death probably due to cerebral metastases.
89 M.D.**	F	papillary	—	—	70.0	5 days	—	—	54.4	Slight uptake occurred in neck, so treated with I^{131}.
92 L.G.**	F	papillary	—	—	91.0	3 mos.	−16	—	94.0	Essentially total thyroidectomy elsewhere, followed by I^{131} therapy.
94 B.H.**	M	papillary	—	8.5	—	6 days	—	3.1	58.8	I^{131} R_1; 85% excretion. Myxedema 3 months.
102 C.N.**	M	papillary	—	—	51.0	2 mos.	—	—	no uptake in metast.	Papillary Ca. of thyroid 2½ years before. Suspected cerebral metastasis.
106 R.O.**	F	papillary	—	—	—	off thyroid 6 days	—	—	97.0	No recurrence. Died of Ca. of breast.
29 M.C.	F	anaplastic	—	—	—	2 mos.	−34	—	85.1	Total thyroidectomy elsewhere.
						6 mos.	—	0.3	96.0	Died in 10 months.
47 G.W.**	F	anaplastic	—	—	66.2	3 days	—	—	101.3	Died shortly thereafter.
67 L.T.	F	anaplastic	+ 4	—	—	8 days	—	—	100.0	Died in 2 months.
70 M.C.	F	anaplastic	—	—	92.0†	3 wks.	—	—	98.4	
						4 wks.	—	—	36.5	Uptake of I^{131} in scapula after total thyroidectomy. Died in 6 weeks.
73 L.D.	F	anaplastic	+ 3	—	35.1	4 wks.	−23	—	88.6	Died of cardiac disease in 6 months.
						4 mos.	myxedema	—	90.1	
108 E.M.	F	anaplastic	—	—	43.5*	8 days	—	—	88.7	Died in 2 months.
114 J.S.	F	anaplastic	—	—	—	2 mos.	—	—	95.0	Total thyroidectomy elsewhere.
						6 mos.	myxedema	—	96.0	
77 E.N.	M	papillary solid	− 8	—	—	5 days	—	—	95.0	No I^{131} in lymph nodes removed 6 months later.
100 J.M.	F	papillary solid	+5	4.3	—	13 days	—	1.7	98.0†	Died in 2 months.
69 S.B.	F	lymphoma	−16	—	—	17 days	—	—	89.5	No uptake of I^{131} in neck.
										No recurrence in 2½ years.
87 A.C.	F	lymphoma	+ 2	—	49.5*	7 days	—	—	80.0*	No recurrence in 6 months.
90 G.D.	F	Hürthle cell	+ 5	8.2	53.7	9 days	− 9	—	93.0	No function in sternal metastases in 9 months.
						6 wks.	−19	0.6	102.0	
						14 wks.	−21	0.0	84.0	

carcinomas an uptake in the metastases was detected after total thyroidectomy. In 2 (Case 33 AK ♂ and Case 70 MC ♀) a total thyroidectomy had been performed, and in the third (Case 46 LT ♀) the surgical excision was almost complete. In none of the anaplastic carcinomas was the uptake sufficient to be of therapeutic value. Hürthle cell, epidermoid, mixed papillary and anaplastic carcinomas or lymphomas could not be induced to collect radioactive iodine by this method.

If in three or four days practically all of a tracer dose of radioactive iodine was not excreted in the urine after a completely total thyroidectomy, the observation suggested that radioactive iodine had been retained in some of the metastatic sites. In 10 of 16 patients with essentially pure follicular adenocarcinoma, the urinary excretion did not approach 100 per cent (Table 4) and myxedema did not develop. *In vivo* counting over the metastatic sites indicated that the tumor was retaining some of the isotope. Of the remaining 6 cases of pure follicular adenocarcinoma, 1 had no distant metastases, 3 were incompletely studied and 2, although classified as follicular in type, were composed of extremely small acini which contained practically no colloid.

Attention has been directed toward the interval of time between the total thyroidectomy and the demonstrable increase in the uptake of radioactive iodine in metastases. An appreciable increase was observed as early as three, four, seven, and fourteen days after total thyroidectomy. Such a change is demonstrated in Figure 2 (Case 68 LA ♂). In 1 patient with a mixed papillary and follicular adenocarcinoma a significant uptake in the metastasis was not demonstrable until six months after the thyroid had been removed. However, frank myxedema did not develop in this patient during the interval in which *in vivo* counting failed to demonstrate an uptake in the metastasis.

The observations thus far suggest that those patients who are most effectively treated with radioactive iodine possess tumors which initially take up some of the isotope and show a prompt increase after total thyroidectomy. Although this increase may be demonstrable soon after thyroidectomy, as time passes, the uptake of iodine usually becomes more pronounced.

An effort has been made to study the histologic changes that take place in metastases following total thyroidectomy. Tissue was obtained either before or at the time of the operation and in 4 cases biopsy specimens were subsequently obtained at various intervals after the total thyroidectomy. Radioautographs were made of the tissue that was removed. Although metastatic lesions show considerable variability within a single lesion or among several lesions in the same patient, and although the sample of tissue cannot be removed a second time for comparison with

itself, the observations on these 4 patients suggest that the metastases have a tendency to develop more resemblance to the normal thyroid than formerly. Indeed some acquire the histologic features of the thyroid of patients with Graves' disease. The probable changes that may have been taking place are illustrated in Figure 3, A and B.

The question arises whether total thyroidectomy, carried out to stimulate the uptake of radioactive iodine in the metastases, may also cause an

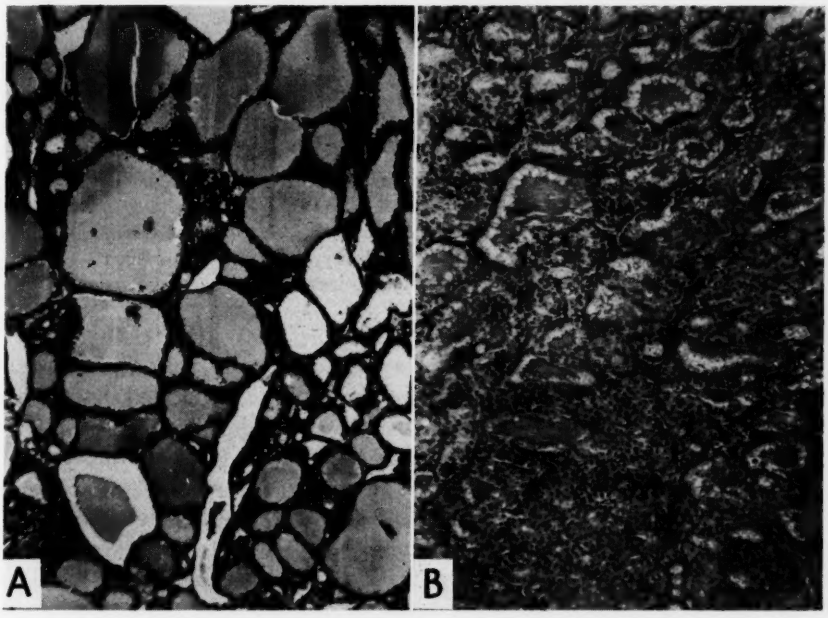


FIG. 3. The histologic changes which may occur in metastases following total thyroidectomy (Case 61, MM ♀). A = Primary lesion of the thyroid; there was no uptake of radioactive iodine (×130). B = A metastatic lesion from the same patient six months after total thyroidectomy; excellent uptake of radioactive iodine (×130). Although it is well known that a metastasis may differ histologically from the primary lesion, similar observations on 4 patients suggested that the metastases have a tendency to develop more resemblance to the normal thyroid than formerly.

increase in the rate of growth of these lesions. One of the patients (Case 79 MS ♀), who subsequently was shown to take up large amounts of radioactive iodine in her metastases, had had roentgenograms made of the right pelvis nine months before she came under our observation (Fig. 4A). During this period of nine months the metastatic lesion in the pelvis had grown moderately (Fig. 4B). Further destruction in the pelvis, apparently at an increased rate, had occurred during 146 days following total

FIG. 4. Changes in the rate of growth of a metastatic lesion in bone before and after thyroidectomy (Case 79, MS ♀). A = Roentgenogram showing metastatic lesion in the inferior ramus of the right pubic bone nine months before total thyroidectomy. B = Roentgenogram of the area at the time of total thyroidectomy. C = Roentgenogram 146 days after total thyroidectomy, illustrating an increase in the rate of growth of the metastatic lesion.

thyroidectomy (Fig. 4C). During the past year the patient has been given a total of 416 millicuries of radioactive iodine in divided doses, and the growth of the tumor now appears to be arrested.

Another patient (Case 61, MM ♀) at the time of total thyroidectomy was found by roentgenograms to have metastatic lesions which were just discernible in the humerus and the pelvis. Six months after total thyroidectomy, the tumor in the humerus had completely replaced a segment of the shaft, and a pathologic fracture had occurred (Fig. 5A). The tumor of the pelvis had reached a diameter of more than 15 cm. Although the rate of growth of the metastases in this patient before thyroidectomy is not known, the rate during the six months following thyroidectomy was extremely rapid. As will be pointed out later, radioactive iodine therapy not only stopped the growth but caused a slight decrease in the size of the lesions. Generally speaking, the impression has been gained that those functioning metastases which have a tendency toward growth following thyroidectomy are those which are most effectively treated with radioactive iodine.

The destruction of the function of the normal thyroid with radioactive iodine

Large doses of radioactive iodine have served as another means of eliminating the normal thyroidal function in patients with carcinoma of the

FIG. 5. Changes in a metastatic lesion in bone after total thyroidectomy and after large doses of radioactive iodine (Case 61, MM ♀). A = Metastatic lesion of the humerus six months after total thyroidectomy. A pathologic fracture had occurred. The lesion was just discernible at the time of total thyroidectomy. B = Same lesion six months later (one year after total thyroidectomy), after 182 millicuries of radioactive iodine in divided doses. C = Same lesion twenty-seven months after total thyroidectomy, and after 434 millicuries of radioactive iodine. Growth of the lesion was curtailed and partial calcification had occurred.

thyroid. It is obvious that if doses of radioactive iodine are large enough, the thyroid can be totally destroyed. This method has been used in our laboratory in 5 patients who had had only a biopsy of the lesion to confirm the diagnosis. At the present time there are several tentative objections to the use of this method when compared to total surgical ablation. First, relatively large doses of radioactive iodine are required to destroy the normal thyroid. This adds to the total amount of radiation received by the patient. Second, the incompleteness of the lethal effect of the first dose may necessitate additional doses. Third and most important, valuable time is lost not only during the period of radiation, but also additional time is necessary for all of the radioactive iodine to disappear so that a new test dose can be given in order to prove total destruction. If multiple doses are required for treatment, the factor of time is further increased. Because there is often a possibility that carcinoma can be completely eradicated by surgery or that the growing lesion may be encroaching on the trachea or esophagus, surgical exploration is indicated in most of the patients. It is at this time that the normal thyroid is removed. The surgical exploration also affords an opportunity to make a histologic diagnosis and an assay of the initial uptake of radioactive iodine by the tumor.

In contrast, it is easier to destroy a small postoperative remnant of normal thyroid than the entire normal gland. Since hyperplastic thyroidal tissue such as that of Graves' disease is more easily destroyed with radioactive iodine than the normal tissue (32, 33), the compensatory hyper-

TABLE 5. THE EFFECT OF THIOURACIL COMPOUNDS ON THE UPTAKE OF RADIOACTIVE IODINE IN METASTASES FROM CARCINOMA OF THE THYROID

Patient					Before administration of thiouracil compound				During administration of thiouracil compound			After thiouracil compound discontinued		
Case No. and initial	Sex	Type of Ca	Interval between observations**	I^{131} treatment during interval (millicuries)	BMR, %	PBI, (γ %)	I^{131} excretion in 72 hours (% of dose)	Duration of drug administration	BMR, %	PBI, (γ %)	I^{131} excretion in 72 hours (% of dose)	BMR, %	PBI, (γ %)	I^{131} excretion in 72 hours (% of dose)
3 T.I.	F	follicular	31 mos.	—	−20	3.0	47.9*	3 days	—	—	84.6	—	—	—
			9 mos.	190	−25†	—	75.5*†	3 days	—	—	74.2*†	—	—	—
			3 days	—	−25†	—	75.5*†	4 mos.	−34	0.0	99.1	—	0.4	75.1
			7 mos.	216	—	—	—	18 wks. Tapazole + thiouracil	−35	—	93.1	—	—	89.8*
7 J.B.	M	follicular	24 mos.	—	−10	3.3	42.6*	14 wks.	−35	0.7	62.5‡	−20	2.4	17.0
			29 mos.	550	− 8	—	84.2*	3 days	—	—	80.9	—	—	57.1
			17 mos.	40	−25	2.0	74.8	3 days	−20	2.2	88.7	—	—	66.8
			6 days	—	—	—	—	8 wks.	−36	0.2	89.3	—	—	63.6
61 M.M.	F	follicular	12 mos.	182	− 1	—	51.3*	3 days	—	—	69.5§	—	—	—
			3 days	—	—	—	—	9 wks.	−19	—	34.9†‡	−10	8.5	11.0
			18 mos.	370	− 5	4.7	52.1	3 days	−10	5.0	98.3	—	—	56.1
68 L.A.	M	follicular	3 mos.	36	−10	—	55.0	3 days	—	—	70.0	—	—	55.0
			12 mos.	422	—	4.9	97.0‖	9 wks.	—	0.2	44.0‡	—	3.2	16.5
72 C.D.	F	follicular	8 mos.	70	−22	1.2	102.0‖	11 wks.	−40	0.0	—	−32	0.2	40.0
79 M.S.	F	follicular	24 mos.	300	−18	3.4	41.3	10 wks.	−33	0.2	35.6‡	—	2.2	9.9
116 L.V.	F	follicular	3 mos.	127	−34	0.0	73.2	5 wks.	—	0.3	—	—	0.2	76.6
34 C.L.	F	papillary	5 mos.	—	−26	—	95.9	7 wks.	—	—	—	—	—	61.7*
92 L.G.	F	papillary	2 mos.	30¶	−16	0.0	89.3	9 wks.	−26	0.0	91.1	—	0.2	96.0
90 G.D.	F	Hürthle cell	4 mos.	—	−20	0.0	94.0	18 wks.	—	—	—	−25	—	93.0
25 H.B.	F	anaplastic	A patient with incomplete thyroidectomy—metastases in skin {tracer dose with thiouracil / tracer dose without thiouracil} no uptake in either.											
47 G.W.	F	anaplastic	A patient with incomplete thyroidectomy.¶ Biopsy while on & off thiouracil; no uptake either time.											

** Interval of time between thyroidectomy and thiouracil observations, or between thiouracil observations.
* Only 48-hour urinary excretion.
† Taking 1 grain of thyroid daily.
‡ In these patients the blocking effect of thiouracil was lost, as illustrated in Figure 7.
§ Incomplete urine collection.
¶ Previous thyroidectomy was medical.
‖ Observation shortly after a large therapeutic dose of I^{131}; transient if not permanent impairment to uptake is often seen soon after therapeutic dose.

plasia in a small remnant of thyroidal tissue apparently makes it more vulnerable to radioactive iodine.

The use of thiouracil compounds as a means of increasing the uptake of radioactive iodine in metastases

Seidlin, Marinelli and Oshry (6) in January 1944 used thiouracil in a patient to control the hyperthyroidism caused by functioning metastases from a carcinoma of the thyroid. On the basis of the urinary excretion of radioactive iodine, this drug blocked the uptake of the isotope in the metastases.

Thiouracil compounds prevent the incorporation of iodine into thyroid hormone; therefore iodine is not stored. The thyroid hormone which has already been made and stored in the tumor continues to be excreted. Eventually the iodine content of the tissue is depleted and when the drug is discontinued, the tumor is more avid for iodine than formerly. Several reports have been made from our clinic as well as from others on the effect of thiouracil on the uptake of radioactive iodine in metastases (8, 13, 22, 24).

In October 1946, thiouracil was given for the first time in our clinic to a patient (Case 7 JB ♂) with functioning metastatic carcinoma of the thyroid, in order to enhance the subsequent uptake of radioactive iodine. This work was carried out under the direction of Dr. Rulon W. Rawson. Although myxedema had not developed in this patient following the total thyroidectomy, it occurred during the administration of thiouracil. When the administration of thiouracil was discontinued, the urinary excretion of radioactive iodine was reduced (Table 5). The successful treatment of this patient with large doses of radioactive iodine then followed.

In the current series, 10 patients were given thiouracil compounds in an effort to induce or increase the uptake of radioactive iodine in metastases after total thyroidectomy (Table 5). Seven of these patients had follicular, 2 had pure papillary and 1 had Hürthle cell carcinoma. All of the first group took up some radioactive iodine after total thyroidectomy. In most of these the uptake was further increased; indeed the retention of radioactive iodine was as high as 90 per cent in 2 patients. One of the 2 pure papillary lesions was moderately affected by thiouracil. One patient (Case 90 GD ♀) with metastatic Hürthle cell carcinoma of the sternum took up no radioactive iodine in spite of eighteen weeks of thiouracil therapy. This patient's total thyroidectomy had not induced function in the metastasis. From our studies it appears that those patients in whom thiouracil is of greatest benefit are those who had some uptake of radioactive iodine in the metastasis after total thyroidectomy. In only 1 instance (Case CL ♀, Table 5) was an uptake of radioactive iodine initiated by the administration of thiouracil.

Although we have not had an opportunity to compare simultaneously the blocking effect of thiouracil on the normal thyroid and on functioning metastatic carcinoma, it has been reported (8, 13) that thiouracil blocks the normal thyroid more readily than it does the metastatic tissue. From studies on several of our patients, it was found that larger amounts of thiouracil were required to block the storage of radioactive iodine in metastases than is commonly used to block the uptake in the normal thyroid. Two hundred milligrams of thiouracil every eight hours was the dose usually given, but in some instances this dosage was doubled in order to produce a satisfactory effect. The biologic difference between patients may be due to large variations in the amount of malignant tissue present

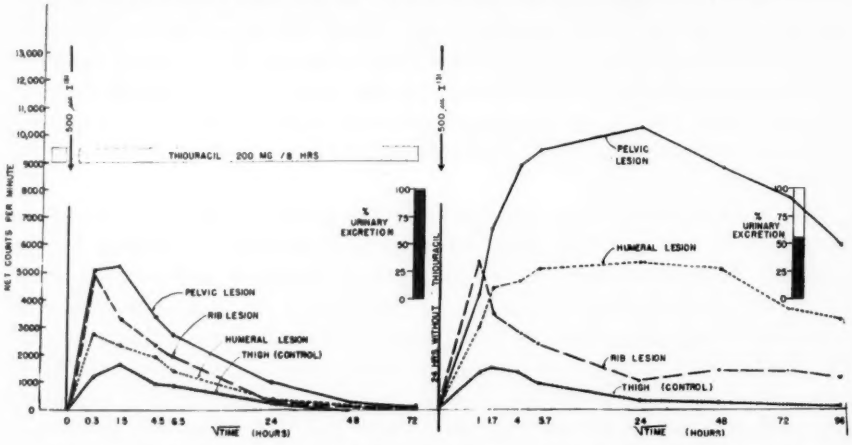


FIG. 6. The use of thiouracil to demonstrate differences in function of several metastases in the same individual (Case 61, MM ♀). Radioactive iodine was not retained in several metastatic lesions while the patient was receiving thiouracil. When thiouracil was omitted, marked variation in the retention of the isotope was noted in the various lesions. The lesion in the rib collected some radioactive iodine but rapidly lost it, as though blocked by thiouracil.

in the body. From the observations, it appears that those patients with extensive functioning metastatic disease required large doses of thiouracil.

Thiouracil has been used to demonstrate the differences of function in several metastatic lesions in the same individual. An example is illustrated in Figure 6 (Case 61, MM ♀). Lesions were present in the pelvis, humerus and rib. There was only a slight temporary retention of radioactive iodine in all three of the lesions while the patient was under the influence of thiouracil. In forty-eight hours all of the isotope had been lost from the lesions. After the administration of thiouracil had been discontinued and a second dose of radioactive iodine given, the lesions in the pelvis and humerus

not only collected radioactive iodine but retained it. At the same time the lesion in the rib only temporarily collected the radioactive iodine and promptly lost it, just as under the influence of thiouracil. The observations indicate that two lesions had a capacity to store iodine while the third did not, and that the collection of iodine could be blocked in the functioning lesions by the administration of thiouracil. Further evidence of the blocking effect of thiouracil on the function of metastases was demonstrated in many of these patients with functioning metastatic disease (Table 5) by the decline in the protein-bound iodine of the serum, by the development of myxedema, and by the increased urinary excretion of a tracer dose of radioactive iodine.

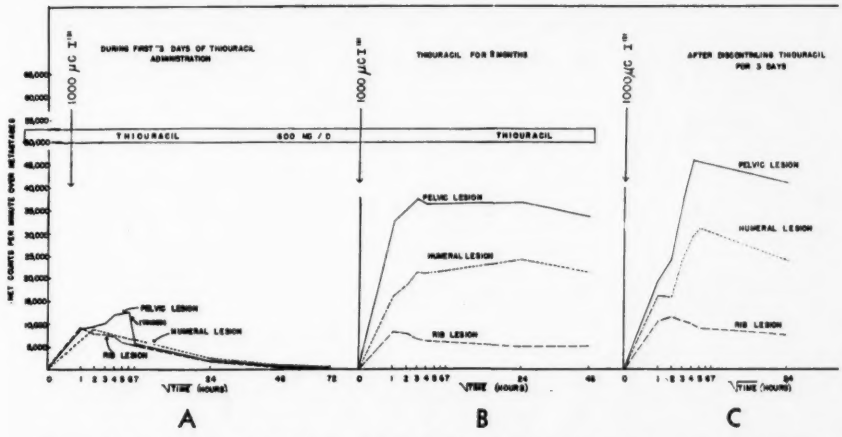


FIG. 7. A decline in the inhibition of the uptake of radioactive iodine produced by the same dose of thiouracil after long continued administration (see Table 5, footnote) (Case 61, MM ♀). A = Inhibition of the uptake of radioactive iodine in 3 metastatic lesions during the first three days of thiouracil administration. B = Incomplete inhibition while receiving the same dose of thiouracil given for two months. C = The uptake of radioactive iodine after thiouracil had been discontinued. A correction was made for the residual radioactive iodine following the second observation.

It has been observed that, although a given dose of thiouracil may be effective for a time, the effect may be lost after long continued administration of the same dose. As illustrated in Figure 7 (Case 61, MM ♀), *in vivo* counting over metastatic sites showed that thiouracil (0.2 Gm. every eight hours) during the first three days of administration blocked the uptake and storage of radioactive iodine in the metastases. Nine weeks later, while the patient was still receiving the same amount of thiouracil, the observations with radioactive iodine were repeated under identical conditions. At this time there was only a slight inhibition to the uptake

TABLE 6. RADIOACTIVE IODINE THERAPY IN PATIENTS WITH METASTATIC CARCINOMA OF THE THYROID

Patient		Location of metastases	Type of Ca	Thyroid-ectomy	Thiouracil compound	I^{131} therapy				Dosage and time at which growth stopped progressing	Remarks
Case No. and initials	Sex					Total dosage (milli-curies)	Total no. of doses	Time interval between 1st and last dose	Largest single dose (milli-curies)		
3 T.I.	F	skull, spine	follicular	total	thiouracil	507	5	28 mos.	100	100 mc 2 yrs.	Two metastases stopped growing for 3 yrs. A new metastasis developed and was uninfluenced by I^{131}.
7 J.B.	M	spine, pelvis	follicular	total	thiouracil	800	18	4 yrs.	166	400 mc 6 mos.	Recovery from neurologic symptoms from lesion in spine; rehabilitated; disease controlled.
40 M.R.	F	liver, adrenal lungs	follicular	subtotal* $+I^{131}$	—	85	2	3 mos.	60	—	Rapid downhill course; only slight uptake of I^{131}. Died of the disease.
59 F.L.	F	skull, sternum	follicular	subtotal*	—	50	1	—	50	—	Rapid downhill course.
61 M.M.	F	humerus, rib, pelvis, spine	follicular	total	thiouracil	730	10	26 mos.	166	200 mc 6 mos.	Patient rehabilitated; skeletal mobility returned to normal.
68 L.A.	M	spine, rib, lungs, pelvis	follicular	total	thiouracil	560	6	17 mos.	195	progressing	Paraplegia shortly after thyroidectomy; no neurologic recovery.
72 C.D.	F	cervical	follicular	total	thiouracil	130	3	11 mos.	57	undetermined	No functioning tissue left; no evidence of regrowth.
79 M.S.	F	pelvis	follicular	total	thiouracil	450	6	15 mos.	150	206 mc 7 mos.	Skeletal mobility markedly improved. Progression of disease controlled following rapid growth.
103 E.N.	F	pelvis, clavicle	follicular	total	—	35	1	—	35	progressing	No appreciable uptake in metastases.
107 M.P.	F	cervical	follicular	total	—	10	1	—	10	—	Small amount of functioning tissue destroyed with I^{131}.
116 L.V.	F	sacrum	follicular	I^{131} + total	thiouracil	215	3	5 mos.	90	progressing	Ineffective uptake of I^{131} in metastasis.
118 A.W.	F	skull	follicular	total	—	62	1	—	62	—	Skull lesion excised.
48 D.Y.	M	cervical	papillary + follicular	total	—	30	1	—	30	—	Decrease in size of cervical metastases.
52 R.C.	F	cervical	papillary + follicular	total	propyl-thiouracil	76	3	—	36	—	Biopsy specimen showed uptake in lymph node. Treatment in progress.
71 M.D.L.	F	cervical	papillary + follicular	total	—	40	1	—	40	—	I^{131} given to destroy slight function in neck after total thyroidectomy. No subsequent uptake. No evidence of recurrence.

TABLE 6—*Continued*

Patient		Location of metastases	Type of Ca	Thyroid-ectomy	Thiouracil compound	I^{131} therapy				Dosage and time at which growth stopped progressing	Remarks
Case No. and initials	Sex					Total dosage (milli-curies)	Total no. of doses	Time interval between 1st and last dose	Largest single dose (milli-curies)		
80 J.S.	M	cervical	papillary + follicular	total	—	30	1	—	30	—	I^{131} given to destroy slight function in neck after total thyroidectomy. No subsequent uptake. No evidence of recurrence.
85 C.A.	M	lung, adrenal, liver, ribs, spine	papillary + follicular	total	—	21	1	—	21	—	I^{131} used to destroy remnant of gland; disease recurred without function. Patient died.
98 E.L.	M	cervical	papillary + follicular	total	—	32	1	—	32	—	Decrease in size of cervical metastases.
10 E.L.	F	cervical, lung	papillary	subtotal* + I^{131}	—	144	4	5 mos.	60	144 mc 5 mos.	Suspected pulmonary metastases regressed.
89 M.D.	F	cervical	papillary	total	—	20	1	—	20	—	Lesion subsequently failed to collect I^{131}.
91 E.D.	M	cervical	papillary	subtotal*	—	35	1	—	35	—	I^{131} given to destroy remnant of normal thyroid.
94 B.H.	F	cervical	papillary	total	—	32	1	—	32	—	I^{131} given because of slight uptake after total thyroidectomy. Observations continue.
97 H.L.	M	cervical, axillary	papillary	—	—	40	1	—	40	—	I^{131} given to destroy normal thyroid. Died of disease.
98 L.G.	F	cervical	papillary	subtotal* + I^{131}	thiouracil	30	1	—	30	—	I^{131} given to destroy remaining normal thyroid. Treatment in progress.
46 L.T.	F	ribs, lung	anaplastic	subtotal*	—	25	1	—	25	—	Undifferentiated metastases took up some I^{131}, but patient rapidly succumbed to disease.
93 H.G.	M	ribs, lung, liver, adrenal, spine	anaplastic	I^{131}	—	102	2	2½ wks.	57	—	I^{131} given for destruction of normal thyroid. Metastases took up no I^{131}.
101 E.M.	F	cervical	anaplastic	total	—	50	1	—	50	—	Rapidly succumbed to disease.
117 O.W.	M	cervical	anaplastic	I^{131}	—	30	1	—	30	—	Rapidly succumbed to disease.
77 E.N.	M	cervical	papillary + anaplastic	total	—	24	1	—	24	—	No follow-up.

* "Subtotal" here means that the normal thyroid was almost totally removed.

of radioactive iodine in the metastases. One explanation for the change in the effect of thiouracil may be that the tumor had become less sensitive to the drug. Another explanation may be that the dose required per unit weight of tumor had become inadequate, due to growth of the tumor tissue.

Although there was no determination of the initial blocking effect during the first three days of thiouracil administration in 3 other patients (Cases 7 JB ♂, 68 LA ♂, and 79 MS ♀), it was found that only 62.5, 44.0 and 35.6 per cent of a tracer dose of radioactive iodine was excreted while the patient was still receiving thiouracil, after fourteen, nine and ten weeks respectively (Table 5). The decreasing effect in the block caused by thiouracil was confirmed by *in vivo* counting over the metastatic lesions in 2 of these patients.

From the evidence thus far acquired, it is impossible to say whether the rate of growth of metastases increased during the time that the patients were receiving thiouracil. Although any single period of thiouracil administration was no longer than two months in these patients, roentgenograms did not reveal a definite increase in the size of metastatic tumors in bone.

Therapeutic doses of radioactive iodine and the effect on metastases

Variations in the method of treating functioning metastases with radioactive iodine have been used (25). The two extremes may be represented by those who give many frequent small doses of radioactive iodine over a considerable period of time and those who wait and give a massive dose of radioactive iodine only after it is found that a large per cent of the dose will be retained. Many variations between these two extremes have been used. The question may be raised whether repeated small doses of radioactive iodine may destroy the functioning capacity of the tumor cells and still not be lethal to them.

Early in our experience, doses up to 50 millicuries were given. Hesitancy to give larger doses was based on a lack of knowledge concerning the radiation damage to other organs or the production of fatal thyrotoxicosis due to massive destruction of the tumor and release of stored hormone. More recently, doses of radioactive iodine as high as 200 millicuries have been given with acceptable risk from radiation hazard. Table 6 illustrates the dosages of radioactive iodine given to the patients treated in this series.

The total mass of functioning malignant tissue is an important consideration. If the mass of tissue is large, the concentration of radioactive iodine per unit weight of tumor may be so low that a lethal radiation effect is not produced.

Our experience has shown that the tumors do not take up the isotope uniformly. Therefore the radiation effect will not be homogenous and sub-

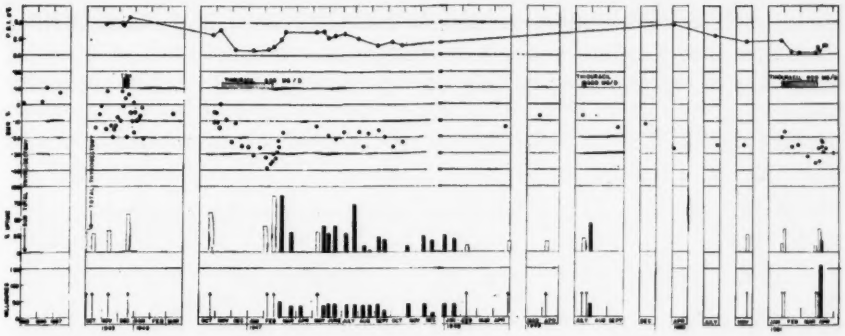


FIG. 8. Carcinoma of the thyroid with skeletal metastases successfully controlled with repeated small doses of radioactive iodine (Case 7, JB ♂). An increase in the uptake of radioactive iodine occurred after total thyroidectomy and after thiouracil administration. Although neurologic symptoms were originally produced by a lesion in the spine, the growth was curtailed, its function was markedly reduced, and the patient was completely rehabilitated. Arrows and light columns represent tracer doses and excretion of radioactive iodine. Dark columns represent treatment doses of radioactive iodine.

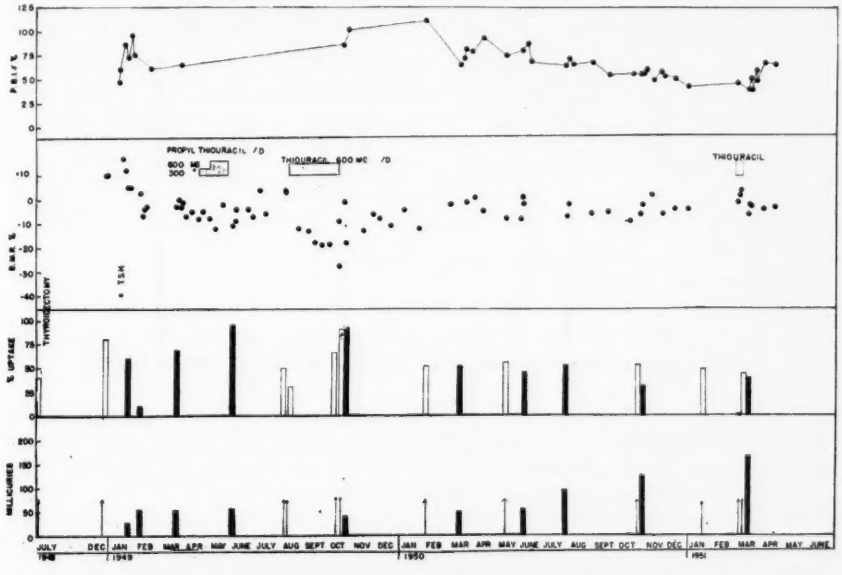

PROPYL THIOURACIL /D
600 MG
300
THIOURACIL 600 MG /D
THIOURACIL
THYROIDECTOMY
% UPTAKE
MILLICURIES
JULY DEC JAN FEB MAR APR MAY JUNE JULY AUG SEPT OCT NOV DEC JAN FEB MAR APR MAY JUNE JULY AUG SEPT OCT NOV DEC JAN FEB MAR APR MAY JUNE
1948 1949 1950 1951

FIG. 9. Carcinoma of the thyroid with skeletal metastases controlled with increasing doses of radioactive iodine (Case 61, MM ♀). Thyroidectomy initiated and thiouracil augmented the uptake of radioactive iodine. A rise in PBI of the serum followed the administration of 166 millicuries of radioactive iodine. This patient, once bedridden owing to a lesion in the pelvis, was rehabilitated (See Fig. 5).

sequent doses will be required after a period of time. Experience further indicates that this interval of time is important because most of a second dose may be excreted if given only a few weeks after the first. The gradually

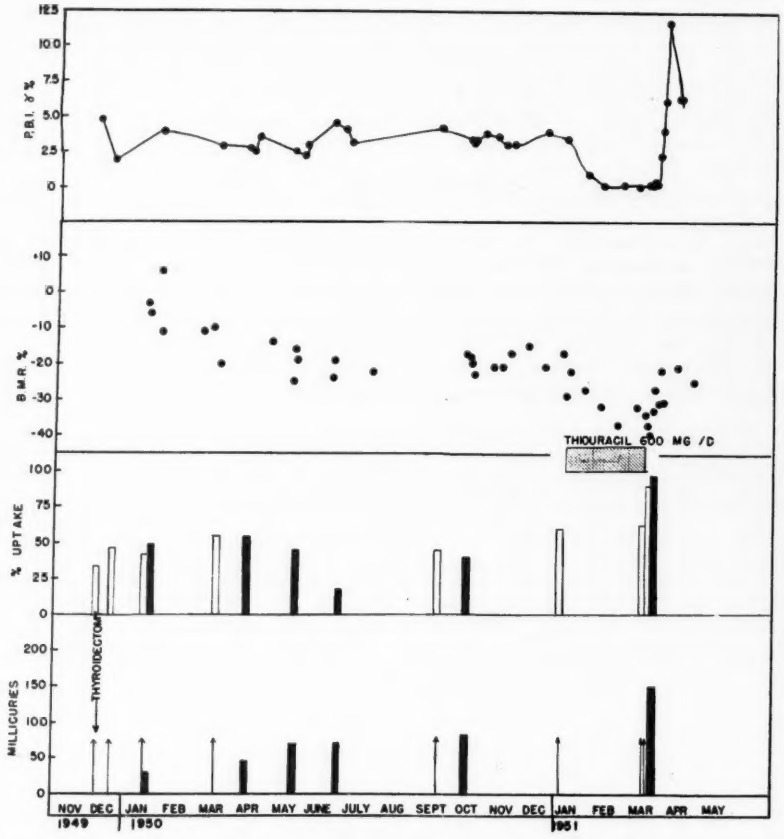

P.B.I. γ %
B.M.R. %
THIOURACIL 600 MG /D
% UPTAKE
MILLICURES
THYROIDECTOMY
NOV DEC JAN FEB MAR APR MAY JUNE JULY AUG SEPT OCT NOV DEC JAN FEB MAR APR MAY
1949 1950 1951

FIG. 10. Carcinoma of the thyroid with skeletal metastases controlled with increasing doses of radioactive iodine (Case 79, MS ♀). Thyroidectomy initiated and thiouracil augmented the uptake of radioactive iodine. A rise in PBI of the serum followed the administration of 150 millicuries of radioactive iodine.

decreasing function of metastases is demonstrated by diminishing uptakes of radioactive iodine in Figure 8 (Case 7, JB ♂).

Trunnell *et al.* (24) and Salter (34) have demonstrated an increase in the protein-bound iodine (PBI) of serum after large doses of radioactive iodine had been administered to patients with functioning metastatic

carcinoma of the thyroid. In several of the patients studied in our series, the serum PBI was determined on several occasions before the treatment dose was given and was subsequently determined at intervals of two to four days following large doses of radioactive iodine. The results are shown in the diagrammatic representation of the course of 2 patients illustrated in Figure 9 (Case 61, MM ♀) and in Figure 10 (Case 79, MS ♀). The

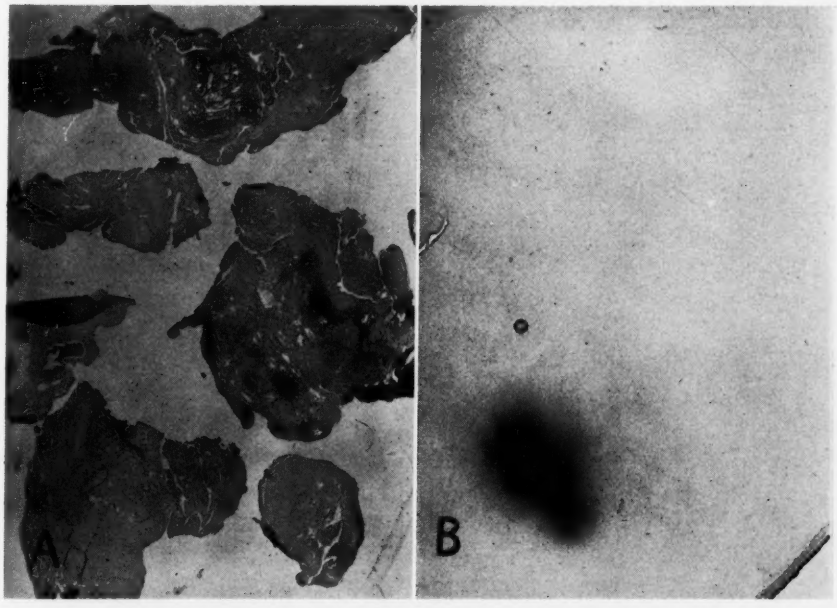


FIG. 11. The destruction and connective tissue replacement of a functioning metastatic lesion. After 280 millicuries of radioactive iodine in divided doses, multiple fragments of the tumor were mounted simultaneously (×10) (A) and a radioautograph prepared (×10) (B). Ghosts of former follicles and connective tissue were present in the numerous biopsy specimens of this lesion. In the lower left corner a small island of functioning tissue remained, as illustrated by the blackening on the radioautograph (B). The cells in this area although viable and functioning showed a marked radiation effect similar to that in Figure 15A. It must be pointed out that some of the connective tissue may have been produced by trauma at the time of former biopsies.

highest level observed in any patient was 11.5 gamma per cent (Case 79, MS ♀). In this patient the observations were as follows: 3.5 gamma per cent before thiouracil was administered, 0.3 gamma per cent toward the end of the period of the administration of thiouracil, and 11.5 gamma per cent nine days after radioactive iodine therapy and eleven days after the thiouracil had been discontinued. In the latter observation, a dose of 150

millicuries of radioactive iodine was given two days after the thiouracil had been discontinued, but only 9.9 per cent of the isotope was excreted in three days.

Histologic studies of serial biopsy specimens of metastases were made in order to observe the effects of radiation on the tumors. The appearance of a follicular adenocarcinoma of the humerus six months after total thyroidectomy is seen in Figure 3 B (Case 61, MM ♀). Multiple simultaneous

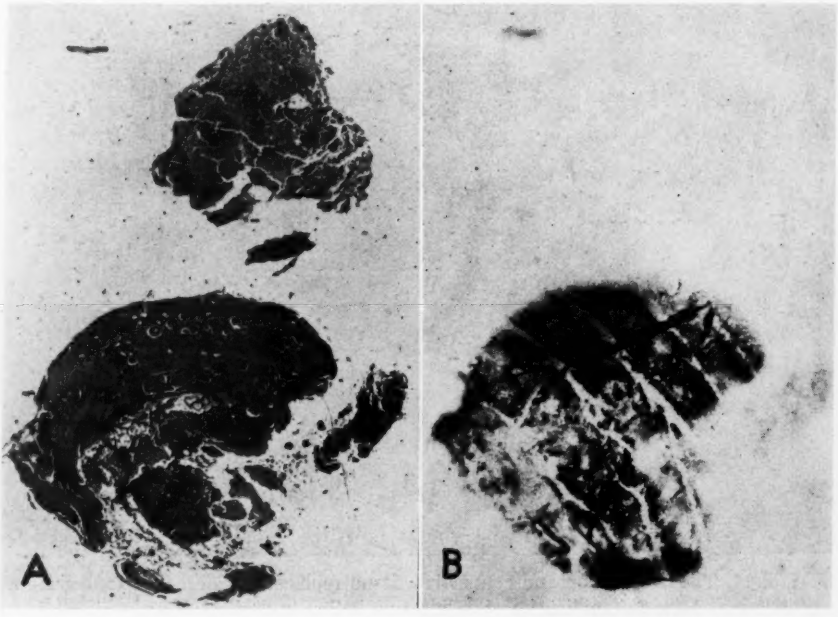


FIG. 12. Different stages of radiation effect found in the same metastatic lesion. A = Two pieces of tissue from the same lesion mounted simultaneously (×25). B = Radioautograph produced by tissues in A (×25) (see text and Figure 13 for explanation).

biopsies of this same lesion (Fig. 11 A, Case 61, MM ♀) were performed after 280 millicuries of radioactive iodine had been given in divided doses (Fig. 9). Ghosts of former follicles were present in some areas of these biopsy specimens. One particular area contained a nest of functioning thyroidal epithelium which caused blackening on a radioautograph after a tracer dose of radioactive iodine (Fig. 11 B).

In another patient with follicular adenocarcinoma (Case 68, LA♂), the biopsy specimen (Fig. 12 A and B) consisting of two pieces of tissue was obtained from a lesion of the spine seven weeks after a dose of 81

millicuries[4] and two weeks after a tracer dose of radioactive iodine had been given. One area of this tumor showed a recent radiation effect (Fig. 13 A), but the radioautograph (Fig. 12 B) of this area revealed no radioactive iodine contained in it. In contrast, the radioautograph of another area (Fig. 12 B) from the same metastasis showed the presence of a small amount of radioactive iodine, presumably from the recent tracer dose. This area showed old fibrosis and damaged acini which were still functioning (Fig. 13 B). It appears that this area did not take up enough of the treatment dose of radioactive iodine seven weeks before, to cause a lethal effect.

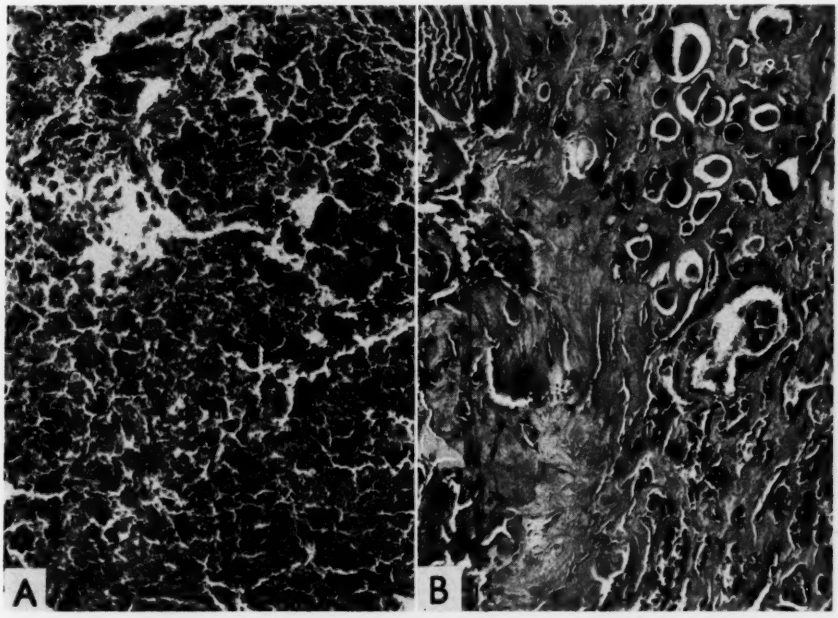


FIG. 13. High magnification of the two pieces of tissue seen in Figure 12A. A. Upper fragment, appearing in Figure 12A, shows an extensive radiation effect resulting from a dose of 81 millicuries of I^{131} given seven weeks previously. This tissue collected no radioactive iodine when a recent tracer dose was given (see Fig. 12B). Although death of the tissue is evident, remnants of acini may be seen in the lower right (×180). B. Lower fragment, appearing in Figure 12A, shows old fibrosis and cellular damage. The thyroid apparently did not collect enough of a treatment dose of radioactive iodine to cause a lethal effect seven weeks before, but later collected enough of a tracer dose to show up in a radioautograph (×95) (see Fig. 12B).

[4] Patient had previously received a total of 365 millicuries in divided doses.

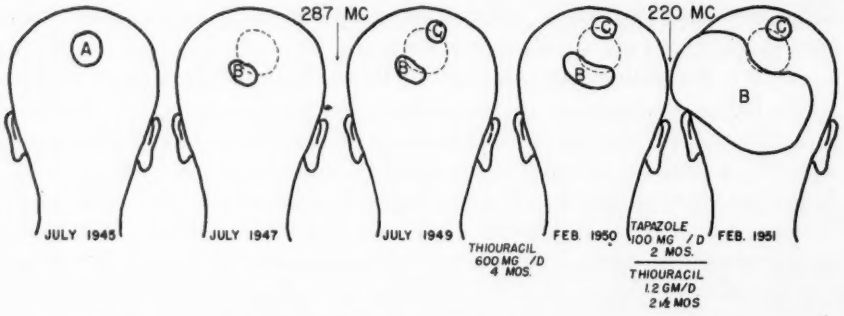


FIG. 14. Differences in the effect of radioactive iodine therapy on the rate of growth of two metastatic lesions in the skull. After the original lesion (A) had been partially excised, two lesions (B and C) appeared at the former site. Lesion C took up radioactive iodine avidly and failed to grow. Lesion B did not take up radioactive iodine and continued to grow. Lesion C appeared shortly after July 1947. Radioactive iodine therapy was instituted after C had appeared.

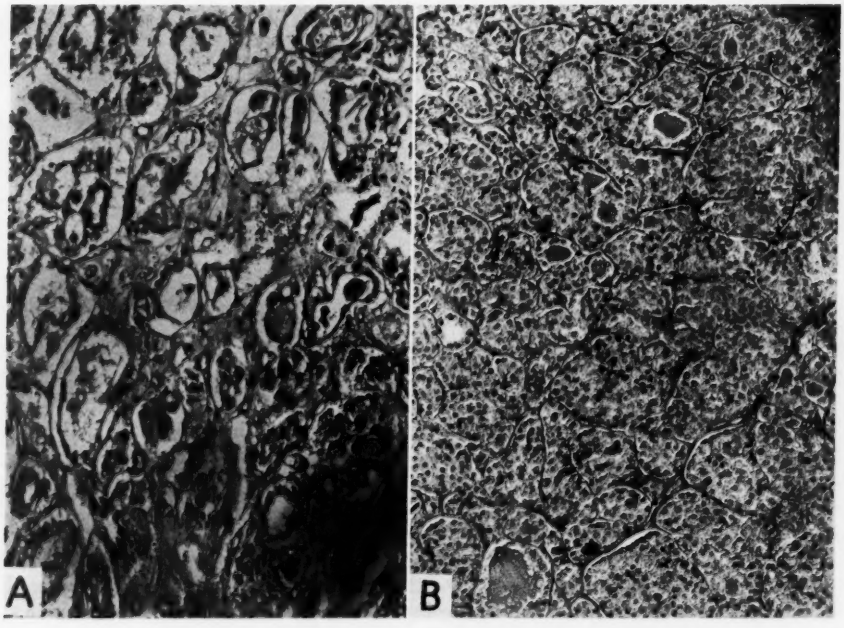


FIG. 15. The histologic evidence of the radiation effect in the metastases illustrated in Figure 14. A = Lesion C in Figure 14. Radiation effect produced by I^{131} (×180). B = Lesion B in Figure 14. This lesion was less differentiated than A and revealed minimal radiation damage (×180).

Observations in another patient illustrate the differences in function and the radiation effects in two metastases in the same patient (Fig. 14; Case 3, TI ♀). After a total thyroidectomy had been performed, a metastatic lesion in the skull took up some radioactive iodine. The lesion at that time measured about 3 cm. in diameter and was subtotally removed. A defect in the skull remained after the partial excision; approximately

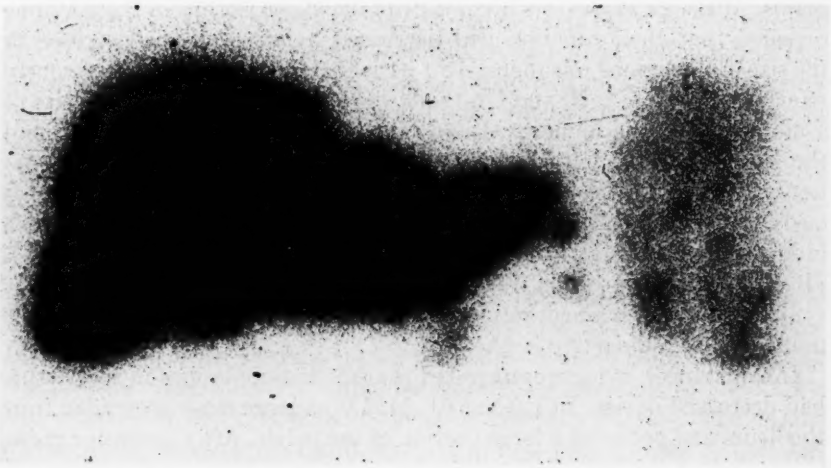


FIG. 16. Radioautograph produced by the two tissues illustrated in Figure 15. The radioautograph on the left was made by the tissue (Fig. 15A) showing previous radiation damage. The radioautograph on the right was made by the tissue (Fig. 15B) showing minimal radiation damage. The two tissue slices that were used were cut to the same thickness, mounted side by side, and simultaneously exposed to the same film for the purpose of comparison.

two years later a nodule appeared at the inferior margin of the defect. Six months later another nodule appeared at the superior margin of the defect. Treatment doses of radioactive iodine curtailed the growth of the superior lesion, but the inferior lesion continued to grow. After four years, the lower lesion began to grow rapidly in spite of large doses of radioactive iodine. During a period of eight months (during six months of which time the patient was receiving thiouracil) the inferior lesion grew until it was approximately one third of the size of the patient's head. One year before the rapid growth of the inferior tumor began, a biopsy specimen of each of the lesions was obtained simultaneously after a treatment dose of radioactive iodine had been given. Extensive radiation effect was found in the superior lesion (Fig. 15 A). The inferior lesion was less well differentiated and showed little evidence of radiation effect (Fig. 15 B). Radioautographs (Fig. 16) were made from both pieces of tissue cut to the same thickness,

mounted side by side and simultaneously exposed to the same film for the purpose of comparison. Much more radioactive iodine was present in the lesion which had previously been partially destroyed than in the lesion which grew.

The effect of radioactive iodine treatment in some patients with functioning follicular adenocarcinoma in bone was studied by serial roentgenograms. Although *in vivo* studies of the uptake of the isotope in these lesions revealed that their function had decreased, relatively little decrease in the size of the lesions was found. In 1 patient (Case 61, MM ♀), previously referred to (Fig. 5 A, B, and C), there was a slight reduction in the size of a metastasis in the humerus. In addition, calcium was deposited around the lesion. Marked improvement in skeletal mobility occurred in 2 patients (Case 61, MM ♀ and Case 79, MS ♀). These individuals previously had been bedridden because of large lesions of the pelvis which interfered with the articulation of the femur. Another patient (Case 7, JB ♂) with a compression fracture of the spine was relieved of neurologic symptoms and remained active five years following bone graft and 800 millicuries of radioactive iodine.

In 2 instances, evidence suggested that metastatic lesions in soft tissues had decreased in size. In 1 (Case 61, MM ♀) a large mass protruded from the ileum and occupied a large portion of the pelvis. After extensive radioactive iodine therapy (740 millicuries), this mass decreased to approximately one third of its former size. In the other patient (Case 10, EL ♀), who had diffuse lymphatic involvement of both lungs from what was originally a papillary adenocarcinoma, the pulmonary lesions are no longer demonstrable by roentgenograms.

Radiation effect on other tissues of the body

Probably the most serious side effects of the radiation from radioactive iodine are changes in the hematopoietic system. Trunnell *et al.* (24) have noted changes in all of the peripheral blood elements. One of their patients, who had received a total of 638 millicuries, succumbed to a fatal pancytopenia approximately one month after a final dose of 250 millicuries had been given. The tumor in this patient took up little to no radioactive iodine. Frantz *et al.* (26) reported 1 patient who had a low platelet count and an alarming leukopenia and anemia. Keating and his associates (35) have observed neutropenia accompanied by monocytosis in a young individual who received a very large dose of radioactive iodine. Seidlin (16) did not observe a decline in the leucocyte count below 3,200 in any patient who had received single doses up to 100 millicuries. Trunnell *et al.* (24) have reported that studies of bone marrow biopsy specimens in 7 cases

revealed a uniform decrease in the total cell count in each instance. The decrease in the circulating lymphocytes has been correlated with an increase in the coproporphyrin excretion in patients receiving large doses of radioactive iodine (36).

Trunnell *et al.* (24) reported 3 normally menstruating female patients under the age of 41, in whom amenorrhea developed after cumulative doses of radioactive iodine ranging from 90 to 458 millicuries. In spite of the fact that the patients complained of hot flashes, the follicle-stimulating hormone titer in the urine was reported to be normal in 2 of these patients. No definite reports could be found in the literature indicating definite renal damage[5] in the human.

Daily blood studies on many of our patients, after large doses of radioactive iodine, have shown that a decline in the actual number of lymphocytes in the circulating blood is more likely to occur than other changes. The greatest observed decline in lymphocytes occurred in a patient (Case 68, LA ♂) who had received 200 millicuries of radioactive iodine in a single dose, of which 16.5 per cent was excreted in seventy-two hours. In this instance, the actual lymphocyte count declined from 750 to 87 by the fifteenth day following administration. The lymphocyte population had returned to the pretreatment level by the thirtieth day. The decline in the actual lymphocyte count was not always proportional to the dose of radioactive iodine given. In 1 patient (Case 61, MM ♀) the actual lymphocyte count declined 40 per cent after retaining 40 per cent of a dose of 27 millicuries. Subsequently this same patient retained 40 per cent of 160 millicuries, but the lymphocytes on this occasion declined only 50 per cent. In the patients thus far treated, there has been no clinical evidence of a bleeding tendency and the blood smears have not shown an appreciable decrease in the number of platelets.

There was an opportunity to observe the ovarian function in 4 premenopausal women who received large doses of radioactive iodine. One patient (Case 61, MM ♀), aged 38, had had normal menstrual periods until the time treatment was begun. After 140 millicuries of radioactive iodine had been administered, the menses stopped. An elevation of the follicle-stimulating hormone in the urine was consistent with ovarian failure. A total of 730 millicuries has been given to this patient; no further menstrual periods have occurred. It is noteworthy that there was a large functioning metastasis in the pelvis. Another patient (Case 116, LV ♀), aged 38, was said to have received 25 millicuries of radioactive iodine in order to destroy the normal thyroidal tissue, before coming under our observation. Amenor-

[5] Miller *et al.* (37) have reported tubular damage in rabbits which had received massive doses of radioactive iodine.

rhea occurred thereafter. The patient subsequently received 250 millicuries, but there was no return of menstrual function.

A patient (Case 10, EL ♀), aged 26, received a total of 144 millicuries; now, two years later, the patient is in her fourth month of pregnancy. A patient (Case 52, RC ♀), aged 15, received a total of 76 millicuries and experienced no menstrual irregularities.

The bromsulphalein test (BSP) was used to study the liver function in

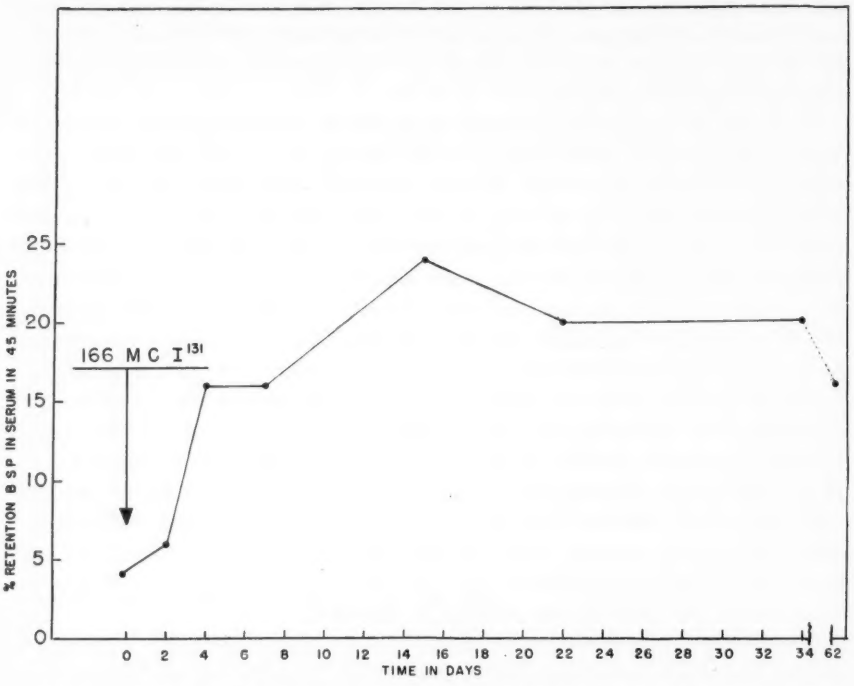


FIG. 17. The rise in the retention of bromsulphalein in the serum of a patient treated with a large dose of radioactive iodine. This patient (Case 61 MM ♀) received 166 millicuries, and excreted 56.1 per cent in the urine in seventy-two hours. The maximal retention occurred at fifteen days. Recovery had not occurred after sixty-two days.

3 patients who received large doses of radioactive iodine. A significant change was observed in only 1 patient (Fig. 17, Case 61, MM ♀). A 4 per cent retention of BSP in forty-five minutes was found in this 41-year-old woman, preceding a dose of 166 millicuries of radioactive iodine (56.1 per cent urinary excretion in seventy-two hours). A significant increase in BSP retention was found by the fourth day. A maximal retention of 24 per cent occurred on the fifteenth day, and recovery was not complete after two months.

The kidney function as measured by phenolsulphonphthalein, urea clearance, urine concentration, urinalysis and nonprotein nitrogen was carefully studied in 1 patient (Case 7, JB ♂) over a period of four years, during which time a total of 800 millicuries of radioactive iodine was given. No significant alterations in the results of these tests occurred in this 65-year-old man.

A marked localized inflammatory reaction occurred in the urinary bladder of 1 patient (Case 79, MS ♀; Fig. 10). A massive metastatic lesion arising in the pelvis and extending to the region of the bladder took up large amounts of radioactive iodine. Although previously the patient had uneventfully received small doses of radioactive iodine, she experienced the onset of urgency, frequency and dysuria twenty-four hours after a dose of 80 millicuries had been given. On cystoscopic examination six weeks following this treatment, Dr. Richard Chute observed an area of erythema over the right side of the bladder in an area adjacent to the metastatic lesion. Ten weeks later, when all urinary symptoms had subsided, this same area was paler than the remaining wall of the bladder. Examinations of the urine during the period of symptoms revealed that there were no organisms in the urine and that only a few leucocytes were present. Twenty weeks following the radioactive iodine therapy, cystoscopic examination revealed the same pale area in the wall of the bladder. A dose of 150 millicuries of radioactive iodine was given five days after this examination. Four per cent was excreted in seventy-two hours (the patient had been on thiouracil). Urinary symptoms began thirty-six hours after the isotope had been given. Cystoscopic examination ten days later revealed marked redness and edema of the same area of the bladder.

SUMMARY

Radioactive iodine has been used to study and in some instances to treat 119 cases of carcinoma of the thyroid. The *initial uptake* of the isotope in these carcinomas and their metastases has been decidedly less than that of the normal thyroid. With this degree of function, radioactive iodine is of little therapeutic value. However, the removal of the normal thyroid has resulted in an increase in the uptake of the isotope in some cases. The advantage thus gained has made radioactive iodine a more useful therapeutic tool, particularly in the follicular adenocarcinomas containing colloid. The administration of thiouracil has been used to increase further the uptake of radioactive iodine in some instances.

Although a few rapidly growing anaplastic lesions have been induced to take up small amounts of radioactive iodine, the uptake in these has not been sufficient to be of therapeutic value. Papillary lesions have been generally regarded as having no function but some lesions so classified

have a follicular component and will take up appreciable amounts of radioactive iodine.

Radioactive iodine therapy has been beneficial in 10 out of 50 patients who were subjected to total thyroidectomy and who had known metastatic lesions remaining. Of the 10 patients, 6 had follicular adenocarcinoma, 3 had mixed follicular and papillary adenocarcinoma, and 1 had papillary adenocarcinoma.

A very few patients with incapacitating metastatic disease have been rehabilitated. The rate of growth of some lesions has been demonstrably curtailed and biopsies of the lesions have shown destruction by the radiation.

A decline in the circulating lymphocytes of the blood is not uncommon after the administration of large doses of radioactive iodine. Although a total dose of 800 millicuries in divided doses and 200 millicuries in a single dose have been given, grave changes in the other circulating cellular elements of the blood did not occur. Ovarian failure and localized inflammatory reaction in the urinary bladder have occurred in patients with functioning carcinomas of the pelvis, apparently owing to the proximity of the lesion containing the isotope. Abnormal retention of bromsulphalein in the serum has reflected damage to the liver in 1 patient.

Acknowledgment

The authors wish to acknowledge the participation of many current as well as former members of the Thyroid Clinic of the Massachusetts General Hospital in the care and study of these patients. *Former members:* Drs. Rulon W. Rawson, Janet MacArthur, Bengt Skanse, William McK. Jefferies, Charles Richards, Thomas Colman, and Henry Koch. *Current members:* Drs. J. Howard Means, Earle M. Chapman, Jacob Lerman, John B. Stanbury, of the Department of Medicine; Drs. Oliver Cope, Edward Hamlin, Jr. and John Raker of the Department of Surgery; Dr. Austin Vickery of the Department of Pathology; and a large technical staff.

REFERENCES

1. Hamilton, J. G.; Soley, M. H., and Eichorn, K. B.: Deposition of radioactive iodine in human thyroid tissue, *Univ. California Publ., Pharmacol.* (#28) **1**: 339–367, 1940.
2. Keston, A. S.; Ball, R. P.; Frantz, V. K., and Palmer, W. W.: Storage of radioactive iodine in a metastasis from thyroid carcinoma, *Science* **95**: 362–363, 1942.
3. Frantz, V. K.; Ball, R. P.; Keston, A. S., and Palmer, W. W.: Thyroid carcinoma with metastases, studied with radioactive iodine, *Ann. Surg.* **119**: 668–689, 1944.
4. Leiter, L.; Seidlin, S. M.; Marinelli, L. D., and Baumann, E. J.: Adenocarcinoma of the thyroid with hyperthyroidism and functional metastases. I. Studies with thiouracil and radio-iodine, *J. Clin. Endocrinol.* **6**: 247–261, 1946.
5. McArthur, J. W., and Cope, O.: The functional capacity of thyroid tumors as judged by radioactive iodine uptake, (abstract) *J. Clin. Investigation* **25**: 929, 1946.

6. SEIDLIN, S. M.; MARINELLI, L. D., and OSHRY, E.: Radioactive iodine therapy; effect on functioning metastases of adenocarcinoma of the thyroid, *J.A.M.A.* **132:** 838–847, 1946.
7. MARINELLI, L. D.; FOOTE, F. W., JR.; HILL, R. F., and HOCKER, A. F.: Retention of radioactive iodine in thyroid carcinomas; histopathologic and radio-autographic studies, *Am. J. Roentgenol.* **58:** 17–32, 1947.
8. RAWSON, R. W.; DOBYNS, B. M.; HILL, R., and FLUHARTY, R. G.: Studies with radioactive iodine on certain functions of benign and malignant tumors of the thyroid. Proceedings Fourth International Cancer Conference, St. Louis, Sept. 1947.
9. RAWSON, R. W.; MCARTHUR, J. W.; DOBYNS, B. M.; FLUHARTY, R. G., and COPE, O.: The functional activity of thyroid tumors, benign and malignant, as gauged by their collection of radioactive iodine, *Tr. Am. Goiter A.* 1947, pp. 187–200.
10. SEIDLIN, S. M.; OSHRY, E., and YALOW, A. A.: Twelve cases of metastatic thyroid carcinoma studied with radioactive iodine, (abstract) *J. Clin. Endocrinol.* **7:** 467–468, 1947.
11. DOBYNS, B. M., and LENNON, B.: A study of histopathology and physiologic function of thyroid tumors, using radioactive iodine and radioautography, *J. Clin. Endocrinol.* **8:** 732–748, 1948.
12. MARINELLI, L. D.; TRUNNELL, J. B.; HILL, R. F., and FOOTE, F. W., JR.: Factors involved in the experimental therapy of metastatic thyroid cancer with I^{131}, *Radiology* **51:** 553–557, 1948.
13. RAWSON, R. W.: Radioactive iodine; its use in studying certain functions of thyroid tumors. Brookhaven Conference Report (Symposium on Radioiodine, July 1948). Technical Information Division, U. S. Atomic Energy Commission, Oak Ridge, Tenn., pp. 4–12.
14. RAWSON, R. W.; MARINELLI, L. D.; SKANSE, B. N.; TRUNNELL, J., and FLUHARTY, R. G.: The effect of total thyroidectomy on the function of metastatic thyroid cancer, *J. Clin. Endocrinol.* **8:** 826–841, 1948.
15. RAWSON, R. W., and SKANSE, B. N.: Radioactive iodine; its use as a tool in studying thyroid physiology, *Radiology* **51:** 525–531, 1948.
16. SEIDLIN, S. M.: Clinical methods and results of radioiodine therapy in metastatic thyroid cancer. Brookhaven Conference Report (Symposium of Radioiodine, July 1948). Technical Information Division, U. S. Atomic Energy Commission, Oak Ridge, Tenn., pp. 106–112.
17. SEIDLIN, S. M.; OSHRY, E., and YALOW, A. A.: Spontaneous and experimentally induced uptake of radioactive iodine in metastases from thyroid carcinoma: a preliminary report, *J. Clin. Endocrinol.* **8:** 423–432, 1948.
18. TRUNNELL, J. B.; RAWSON, R. W.; MARINELLI, L. D., and HILL, R.: The effect of thyroid stimulating hormone on the function of human normal and malignant thyroid tissue, (abstract) *J. Clin. Endocrinol.* **8:** 598, 1948.
19. DOBYNS, B. M.; SKANSE, B. N., and MALOOF, F.: A method for the preoperative estimation of function in thyroid tumors: its significance in diagnosis and treatment, *J. Clin. Endocrinol.* **9:** 1171–1189, 1949.
20. FITZGERALD, P. J., and FOOTE, F. W., JR.: The function of various types of thyroid carcinoma as revealed by the radioautographic demonstration of radioactive iodine (I^{131}), *J. Clin. Endocrinol.* **9:** 1153–1170, 1949.
21. RALL, J. E.; KEATING, F. R., JR.; POWER, M. H., and BENNETT, W. A.: The distribution of radioiodine in a patient with metastatic adenocarcinoma of the thyroid, *J. Clin. Endocrinol.* **9:** 1379–1391, 1949.

22. Rawson, R. W.; Marinelli, L. D.; Skanse, B. N.; Trunnell, J. B., and Fluharty, R. G.: Radioactive iodine: its use in studying certain functions of normal and neoplastic tissues, *Cancer* **2:** 279–292, 1949.
23. Seidlin, S. M.; Rossman, I.; Oshry, E., and Siegel, E.: Radioiodine therapy of metastases from carcinoma of the thyroid: a six-year progress report, *J. Clin. Endocrinol.* **9:** 1122–1137, 1949.
24. Trunnell, J. B.; Marinelli, L. D.; Duffy, B. J., Jr.; Hill, R.; Peacock, W., and Rawson, R. W.: The treatment of metastatic thyroid cancer with radioactive iodine: credits and debits, *J. Clin. Endocrinol.* **9:** 1138–1152, 1949.
25. Frantz, V. K.: In discussion of (26), *Tr. Am. Goiter A.* 1950, p. 359.
26. Frantz, V. K.; Larsen, W. G., and Jaretzki, A.: An evaluation of radioactive iodine therapy in metastatic thyroid cancer, *Tr. Am. Goiter A.* 1950, pp. 345–352, and *J. Clin. Endocrinol.* **10:** 1084–1091, 1950.
27. Kaae, S., and Peterson, O.: The treatment of thyroid carcinoma with radioactive iodine, *Acta radiol.* **33:** 539–556, 1950.
28. Weinberg, S. J.; Fink, R. M.; Fink, K., and Packer, G. L.: Metastatic adenocarcinoma of the thyroid with elevated basal metabolism: radio-iodine studies, *Ann. Int. Med.* **32:** 1207–1224, 1950.
29. Graham, A.: A study of the physiological activity of adenomata of the thyroid gland in relation to their iodine content as evidenced by feeding experiments on tadpoles, *J. Exper. Med.* **24:** 345–361, 1916.
30. Means, J. H.: The Thyroid and its Diseases. Philadelphia, J. B. Lippincott Co., 1937, p. 482.
31. Von Eiselsberg, A.: Ueber physiologische Function einer im Sternum zur Entwicklung gekommenen krebsigen Schilddrüsenmetastase, *Arch. f. klin. Chir.* **48:** 489–521, 1894.
32. Freedberg, A. S.; Blumgart, H. L.; Kurland, G. S., and Chamovitz, D. L.: The treatment of euthyroid cardiac patients with intractable angina pectoris and congestive failure with radioactive iodine, *J. Clin. Endocrinol.* **10:** 1270–1281, 1950.
33. Chapman, E. M., and Maloof, F.: Unpublished data.
34. Salter, W.: The metabolic circuit of the thyroid hormone, *Ann. New York Acad. Sci.* **50:** 358–376, 1949.
35. Keating, F. R., Jr.: Personal communication.
36. Koch, H. J., Jr.; Talbot, T. R.; Bernstein, M.; MacKinnon, J., and Elias, C.: The effect of isotopic (I^{131}) radiation and X-radiation on the total coproporphyrin excretion in humans, *Tr. Am. Goiter A.* 1950, pp. 185–209, and *J. Clin. Endocrinol.* **10:** 1029–1053, 1950.
37. Miller, E. R.; Lindsey, S., and Soley, M. H.: Effect of large doses of I^{131} in experimental animals. Unpublished data cited by Soley, M. H., and Miller, E. R.: Treatment of Graves' disease with radioactive iodine, *M. Clin. North America* **32:** 3–17, 1948.

FOODS AND THE GENESIS OF GOITER*†

MILDRED B. FERTMAN, M.A., M.D.
AND GEORGE M. CURTIS, PH.D., M.D.

From Stanford University Medical School, San Francisco, California, and the Department of Research Surgery, Ohio State University, Columbus, Ohio

THE nutritional complex eventually resulting in the development of goiter is composed of one or more of three differing elements, *deficiency*, *excess*, or *toxicity;* moreover, more recent studies have even suggested two other factors, antagonism as well as synergism. Among these the role of deficiency is best known. Nearly world-wide experience indicates that iodine lack is concerned in the production of at least one form of thyroid disease, endemic goiter, although other circumstances may contribute to a varying degree. The iodine-deficiency hypothesis alone, however, fails to explain satisfactorily several situations which are still enigmatic. Thus, there is the puzzle of certain isolated seacoast regions where the iodine intake is presumably high and yet simple goiter is prevalent; or the more baffling observations concerning cretinism, which over-runs certain areas endemically while shunning others.

It is particularly owing to such exceptions, and also perhaps to certain chance associations, that efforts are constantly being made, despite the well grounded hypothesis of iodine lack, to isolate as yet unknown or unconfirmed etiologic factors in simple goiter and to disclose the causes of other thyroid disease as well. In the complicated domain of medicine, there are usually to be found new shock absorbers, occasional synergists and many antagonists.

Although many ideas have been advanced and tested, only to fall by the wayside, a few suspected goitrogenic agents have been receiving increasing attention in the developments of animal experimentation as well as in the application of more recent physiochemical studies. Among these agents or their related compounds it has been hoped there could be discerned those elusive substances present in certain foods which may prove responsible for thyroid hyperplasia and perhaps even for subsequent nodule formation. One goitrogenic substance has thus far been isolated from foodstuffs (1).

Received for publication July 25, 1951.

* Read at the Annual Meeting of the American Goiter Association, Columbus, Ohio, May 24, 1951.

This article will be included in the bound volume of the "Transactions of the American Goiter Association" published by Charles C Thomas, Publisher, which will be available for sale early in 1952.

† Aided by a Grant from The Comly Fund for Research of the Ohio State University.

PLANT FOODS AND FOOD SUBSTANCES

The food receiving the greatest attention thus far as concerns the origin of goiter has been the widely used vegetable, cabbage. "Mir graut vor Kraut" was the conclusion of Wagner-Jauregg and Koch (2a). It now appears that the etiologic role of "Kraut" has been greatly overrated (1) (3). Where *balance* is the keynote of nutrition, cabbage and its relatives may prove even desirable. The byword might better be expressed: "Mir graut vor *zu viel Kraut*" and we might add, "und *zu wenig Jod!*"

Chesney, Clawson and Webster in 1928 drew world-wide attention to cabbage when they noted the development of large goiters, in spite of an adequate iodine intake, in laboratory rabbits maintained on a daily ration of this vegetable (4). These rabbits had been singled out for studies of a different nature. Further investigation here (5) and abroad (2, 6) followed, and for the most part similarly demonstrated that not only certain varieties of cabbage, but also other members of the genus *Brassica* may have similar effects upon the thyroid gland.

Goitrogenic activity seems to be a property of the *seeds* of all the Brassicae (7) but its role in the edible portions such as the blossoms, leaves and roots (2), has not been as firmly established (1). The goitrogenic agent, vinyl-thio-oxazolidone, isolated from various Brassica seeds, has thus far been demonstrable in the edible portions of only rutabaga and white turnip (1).

Using the Brassica-seed diet, striking results have been obtained in rats given adequate iodine rations (7). Kennedy and Purves found that during the first seven days the thyroid loses the greater part of its colloid. There is considerable hypertrophy of the epithelium, the gland as a whole remaining unchanged in size. During the second week there occurs a rapid increase in the weight of the entire gland. At fourteen days advanced hypertrophy together with hyperplasia is observed, and complete loss of colloid. At the end of the third week the changes are at a maximum. Thereafter, a slow thyroid growth continues, paralleling that of body growth in the rat. Colloid, absent from the fourteenth day, reappears in significant amounts in glands examined subsequent to the fifty-sixth day. Since no progressive accumulation of colloid occurs thereafter, it is assumed that the thyroid at this period has attained physiologic equilibrium (7).

Along with these thyroid changes there have been observed hypertrophy of the adrenal cortex (7), delay in development of the ovaries of immature females (7), and histologic changes in the pituitary (7, 8a). The changes in the pituitary (8), particularly, are of fundamental significance in the understanding of the mode of action of Brassica seeds in the initiation of thyroid enlargement.

Of the edible Brassicae, the following have been reported to produce goitrogenic activity in experimental animals:

Brussels sprout (5)	rape (2)
cabbage, white and red (2, 4, 5)	rutabaga (1)
cauliflower (2, 5)	savoy (2)
kohlrabi (2)	turnip (2, 9)

Other vegetables reported to be goitrogenic in animals when given in sufficient amount are (2):

bean	lettuce
beet	pea
carrot	peanut
lentil	

and also soybean and soybean products (2, 10–14). Greer, who has not been able to confirm the goitrogenic effect of soybean, suggests that iodine deficiency may not always have been satisfactorily excluded as a cause (15).

It was difficult to explore the possible goitrogenic effects of foodstuffs in man until Greer and Astwood conceived of using radioactive iodine uptake as an indicator (3). Unexplained and uncontemplated were the results with cabbage, the vegetable which originated a widespread quest for food goitrogens. Varieties of cabbage, by this method at least, failed to show any decided antithyroid activity in man (3). A most striking effect occurred, however, with rutabaga, a close relative. A moderate effect was noted with another member of the same genus, white turnip (3, 15), and also (15) with the less closely allied foods:

carrot	spinach
peach	strawberry
pear	

Certain other vegetables and fruits originally thought to have shown antithyroid activity (3), are now believed by subsequent experiments to be questionable in this respect (15). These include:

apricot	honeydew melon
beet	lettuce
celery	orange
filbert	pea
grapefruit	peanut
grape	string bean
green pepper	walnut

Difference in lots and possibly also in individual susceptibility may have been responsible for the occasional varying results obtained with any given vegetable (3). Range of iodine content of a foodstuff was not thought to be a significant factor since the amount of iodine contained in any food,

with the possible exception of seafood, was considered too low to influence the uptake of radioactive iodine sufficiently.

Negative results were obtained in human subjects (3, 15) with the following Brassicae:

broccoli	mustard
cauliflower	radish

In addition, the following vegetables or fruits showed no antithyroid activity in man (3, 15):

almond	olive
apple	onion
banana	pineapple
black and lima bean	potato
blackberry	rice
corn	rye
cucumber	squash
date	tangerine
loganberry	tomato
mushroom	

Effects of heat and other physical agents upon the antithyroid property of these foodstuffs vary (1, 3, 12, 13, 14, 16). It has been reported that if cabbage is steamed and kept exposed in the moist state to air, the goitrogenicity is not affected (16). On the other hand, drying, with (2) or even without (16) cooking, has been shown to destroy this property. In human subjects the strongest antithyroid effect of rutabaga was observed when raw chunks were fed; a lesser effect was noted when the rutagaba was pureed, whereas the result was negative when it was cooked (3).

Observations concerning the effects of heating and moisture are now explained, at least for one known goitrogenic agent, vinyl-thio-oxazolidone, isolated from Brassica seeds (1). This substance does not occur free in an active form in the plants. Loss of goitrogenicity brought on by heating or by grinding the food with alcohol is not due to destruction of thio-oxazolidone but rather to the inactivation of an enzyme essential for its liberation. With sufficient hydrolysis, enzymatic liberation of thio-oxazolidone ensues. Thus, if Brassica seeds are first finely ground or steeped in aqueous solution, even prolonged boiling can no longer affect their goitrogenic activity (1).

The goitrogenicity of soybeans has been reported as only partially inactivated by heat (13, 14), and that of unprocessed soybean flour is apparently destroyed not only by steam but also by fat solvents (ether or acetone) (12). On the other hand, it has been observed that fat solvents such as ether, ethanol or chloroform do not remove the goitrogenicity of commercial soybean meal (13).

The effect of iodine administration upon the goitrogenic property of foodstuff has been of especial importance in understanding the modes of action of the active agents and in relating these agents to known goitrogenic compounds. Recent investigations have shown that iodine in small amounts will completely prevent the development of thyroid hyperplasia due to soybean rations in rats (12–14) and in chicks (13). Halverson and his associates have found that the quantity of iodine necessary is minimal (14), so minimal in fact that Greer (15), who has not been able to confirm the goitrogenicity of soybeans *per se*, suggests that perhaps iodine deficiency may not have been sufficiently excluded as a cause in these experiments. On the other hand, the iodine requirement of animals maintained on soybean-containing rations is reported as slightly increased (14), or even possibly doubled (12).

The administration of iodine will not prevent the development of thyroid hyperplasia resulting from a Brassica seed diet (7) but may moderate the effect (17). Diiodotyrosine is equally useful in diminishing the goitrogenic action of a rape-seed diet upon rats, but will not abolish this action even in large doses (17). *Thyroxine, however, given daily is wholly effective.* From these findings it may be concluded that the positive goitrogenic agent of the Brassicae acts through interference with the synthesis of thyroxine by the follicle cells of the thyroid gland (17) and further, that it operates in similar fashion to drugs such as thiouracil and its derivatives.

Other studies not only substantiate such similarity but also show that the goitrogenic agent of Brassica seed probably acts though the medium of the thyrotopic hormone of the pituitary (8). In the first place, as Griesbach and his associates have demonstrated, upon feeding Brassica seed to rats there ensues thyroid hyperplasia with concomitant changes in the anterior pituitary, such as an increase in the basophil cells (presumably the source of thyrotropic hormone) and the appearance of "signet cells" similar to those seen following thyroidectomy (8a). After reaching a maximum at fifty-six days, however, the basophils apparently approach a more normal picture (8a) at a time when colloid reappears (7, 8a) in the hyperplastic thyroid. Secondly, the *hyperplasia previously induced by Brassica-seed feeding rapidly regresses after hypophysectomy*, despite continued administration of Brassica seeds (8b). Thirdly, the thyrotropin content of the serum of animals on a Brassica-seed diet is raised to the same level as that induced by thyroidectomy (8c).

Thus, to produce thyroid hyperplasia, the Brassica-seed diet requires the intervention of thyrotropic hormone (8b). The chain of events might therefore be similar to that following administration of the antithyroid drugs of the aminobenzene and thionamide series (18, 19): thyroid enlargement is produced by interference with formation of thyroxine; the pitui-

tary in turn is stimulated to increase its output of thyrotropic hormone; and this leads to further hypertrophy, and to hyperplasia of the thyroid gland (15).

The goiter resulting from overfeeding with cabbage may differ from that effected by Brassica seeds and rutabaga (1). Iodine administration has been reported to prevent completely the development of "cabbage goiter" (4b), whereas it only partially inhibits the Brassica-seed goiter (1, 7, 17). Moreover, antithyroid activity, as demonstrated with Brassica seeds in radioiodine uptake studies, so far has not proved significant with differing varieties of cabbage in rats or man (1, 3, 15). Consequently it was to be expected that either (a) there is no goitrogenic agent in cabbage and that some other factor, such as iodine deficiency for one, may provide the stimulus for "cabbage goiter", or (b) the goitrogenic principle in cabbage is effective or present only under certain circumstances. Vinyl-thio-oxazolidone, the goitrogenic agent in Brassica seeds, has not been demonstrated in cabbage (1).

There may be a seasonal and species difference in the goitrogenic content not only of cabbage but of rutabaga as well (15), and possibly of other foodstuffs. This difference may depend, as Greer has suggested, on a variation of soil, sun, rainfall and reducing substances present in the food (15). The influence of soils, water and season has long been suspected and long sought out in the earlier investigations on goiter, and has been attributed to such characteristics as, for example, the iodine poverty of soils, infectious agents in water and insufficient ultraviolet irradiation. Now there appear to be other possible predisposing or causative factors as well, which may operate through the environment of our flora and fauna. There would thus appear to be no simple equation to account for the development of all goiters.

FOODS OF ANIMAL ORIGIN

Less is known concerning the goitrogenic effects of animal products. Some antithyroid effect was at first noted in man from such foods as milk, custard and liver, by use of the method of radioiodine uptake (3). Further investigation however appears to have cast some doubt on the significance of these specific results (15).

Liver, one of the foods tested by the principle of radioactive iodine (I^{131}) uptake (3, 15), has been reported to be "antithyrotoxic" in man (20). It may display goitrogenic activity in animals (21, 22). Rats fed a diet containing soybean flour, millet seed and liver showed enlargement of the thyroid (21). The size of the gland thus encountered far exceeded that observed when dried beef was substituted for liver or the liver was extracted with alcohol. Again, similar enlargement ensued upon addition of

the residue from the alcohol extract of liver to the diet containing dried beef (21). Soybean flour was kept constant in these varying diets and most likely, therefore, could not have influenced the differences observed. A positive goitrogenic action has also been ascribed to liver by Remington in an experiment designed to exclude iodine deficiency as a factor (22).

Early investigations yielded evidence that under certain circumstances enlarged thyroid glands may be associated with high protein diets (15). "Under certain circumstances" should be underlined. Again, other factors necessary to thyroid health or causative in thyroid disease may have played the etiologic role. The availability of a large amount of protein in the absence of other needed substances might have upset the normal balance.

That *balance* may be an important consideration received support from a study demonstrating the relative proportion of animal fats and proteins to carbohydrates as at least a contributing factor in the production of goiter (23). This ratio, it would appear, was a determining factor as to where goiter would develop in Oberbaden, an area in which goiter is endemic (23). Careful analysis of the diets revealed that fats and proteins were consumed more copiously in "goitrous" that in "nongoitrous" households. Carbohydrates in the form of bread and pastries, and vegetable fat such as margarine, were used more extensively in the "nongoitrous" households. Seafood, which would have been a suitable source of iodine, was consumed only negligibly in both the "goitrous" and the "nongoitrous" households. No significant difference could be shown in the total iodine content of the foodstuffs of the two groups (23). It would be interesting to know whether there were any dissimilarities in vegetable consumption among the two groups.

In rats receiving a diet corresponding to that of the "goitrous" households, enlarged thyroids developed with microfollicular structure, high epithelium and but little colloid (23). The eosinophil cells of the anterior hypophysis were increased; the iodine content of the thyroid was reduced and the urinary iodine was augmented. Rats receiving food representative of the diet in "nongoitrous" households showed none of these changes. However, when the fat and meat portion was increased five times, at the expense of carbohydrate, again thyroid hyperplasia, with attendant histochemical and associated hypophyseal changes, was encountered. A sufficient relative increase of the carbohydrate portion in the diet of "goitrous" households prevented thyroid and hypophyseal changes in rats. Moreover, sufficient iodine was found to prevent the sequelae of meat-and-fat-rich diets (23).

From these findings one may surmise that in areas of endemic goiter the use of relatively large quantities of animal fat and protein can further aggravate a situation of iodine lack. This hypothesis remains to be con-

firmed. There is confusion regarding the role of high proteins. As for fats, Remington (24) and Rogers and Eckstein (25) have shown that dietary fat (24, 25), regardless of type or amount (24), has no demonstrable influence upon the weight or structure of the thyroid gland.

Lack of protein, rather than its overabundance, has been considered in one report to be an etiologic factor in the increased incidence of goiter in Germany following World Wars I and II. The reason given is the lesser frequency of goiter observed among the socially higher strata (26). However, protein can hardly have been the only consumed food substance which distinguished rich from poor, particularly in a war-ravaged nation.

In patients suffering from hyperthyroidism (toxic goiter), a uniform tendency to select a diet containing a subnormal number of protein calories has been observed (27). When given a diet containing the normal percentage of protein, the patient with toxic goiter tends constantly to revert to his own preference for a subnormal protein intake. Whether this tendency is one of organic self-preservation in the wake of disease or an individual habit pattern conducive to the development of toxic goiter, might be important to investigate.

VITAMINS

It comes as no surprise that one or other of the vitamins has been thought to play some role in the normal activity of the human thyroid gland. Vitamins are often regarded as an invincible shield by the public and are a continued source of renewed interest to the research minded physician. They have well known properties vitally needed by the human body and may possess, in addition, as yet unrevealed qualities of equally great significance.

At least one vitamin may be involved in those complex mechanisms which eventually lead to the elaboration of the thyroid hormone. With avitaminosis A, hypertrophy of the thyroid of the female rat and atrophy in the male have been encountered (28). It has been reported that thyroid weight is increased (28, 29) in vitamin A deficiency; that the iodine metabolism, as demonstrated with radioactive iodine, is abnormal (29); and that the rate of formation of thyroxine is decreased (29).

In hypervitaminosis A, on the other hand, there may ensue a reduction in thyroid weight (30) and a diminution of the colloid within the thyroid follicles (31). In addition, increases in the pituitary, muscle and serum protein-bound iodine levels and a marked decrease in the hepatic iodine have been noted (32). These changes suggest the following trend of events (32): Because destruction of thyroxine by the liver is reduced, there is an increase in the circulating thyroxine. Decreased thyrotropic hormone formation results, with an eventual diminution in both the weight and iodine content of the thyroid gland (32). Since the effects on iodine levels are

more noticeable when vitamin A is fed alone rather than in conjunction with iodine, it has been concluded that the vitamin does not reduce thyrotropic secretion by formation of vitamin A-iodide or thyroxine compounds (32).

Vitamin A given in massive doses to patients with hyperthyroidism results in a rapid decrease of the basal metabolic rate, an increase in the level of the blood cholesterol and an improvement of the symptoms (33).

That synergism, or even antagonism, may play a role in the production of goiter is indicated by the work of Bruman and Blomberg on Vitamin D (34). They suggest that the contradictory results obtained by different investigators with acetonitrile are due to a seasonal variation, the antithyroid activity of this substance being most apparent in the winter. The seasonal variation is attributed to a relative vitamin deficiency, for the thyroid hyperplasia ordinarily produced by acetonitrile in winter animals was found to be prevented by either ultraviolet rays or vitamin D. Synergistic action between vitamin D and the thyroid hormone is thus suggested (34). This hypothesis, if substantiated, may also explain differences observed in goitrogenicity of winter and summer cabbage (5).

There have also been attempts, with varying results, to implicate vitamin B (35, 36) and vitamin C (37–39). Harris and Remington came to the conclusion that vitamin B_1 cannot be substituted for iodine in preventing low-iodine goiter (36). Likewise, the role of vitamin C in the development of goiter has been questioned. Attempts to show that there is no protection against goitrous changes in a scorbutic diet (37) have been unconfirmed (38). Although large thyroids have been reported to result from diets deficient in vitamin C (37, 39), it is now suggested that the increased size of these glands may have been chiefly due to extravasated blood (15).

In an investigation of "mass" goiter among Red Army soldiers, results indicated that vitamin supplements in the form of cod liver oil, lemons with the peel present, and yeast, would effectively involute goiter in 9 out of 10 subjects, improvement being noted as early as the third day in 1 patient (40). Either customary circumstances, or improved sanitary conditions and leisure, or administration of calcium iodide apparently had no significant advantage. Might the calcium have counteracted the effect of the iodine in the calcium iodide? Was the thyroid enlargement simple goiter?

SPECIFIC ELEMENTS

Iodine has been securely established as a most important element in the normal function of the thyroid gland. Several other chemical substances or minerals which may or may not be found in foods have been investigated for goitrogenicity, or conversely, for their value in the prevention of endemic goiter. First, there are the other closely related halogens which

might, hypothetically at least, replace iodine in the synthesis of thyroid hormone. Until recently, bromine has not received much consideration (41, 42). Thyroids of mice given potassium bromide show an increased secretory activity. Moreover, these thyroid glands, when transplanted, induce an accelerated metamorphosis of tadpoles (41). Another experiment with the halides demonstrates that each of the non-iodine halides (bromine, chlorine and fluorine) may cause distinct augmentation of the goitrogenicity of the thiouracils, fluoride exerting the greatest action (42).

Such a role of fluorine, at least, may be consistent with findings which correlate the distribution of endemic goiter in the Punjab (India) and in England with the geologic distribution of human dental fluorosis (mottled enamel) (43). For example, dental fluorosis has been noted among school children in two areas of high goiter incidence in Somerset, whereas it was absent in an adjoining nongoitrous area (43). Experimental work with fluorine seems to show that this substance will induce in the rat hypoplasia of the thyroid epithelium and an altered staining reaction of the colloid (44). Extensive clinical work has led May to advocate fluorine in the treatment of toxic goiter (44).

Steyn's investigation in South Africa seems further to bear out the possibility of a fluorine-iodine antagonism and shows that at least in some areas of endemic goiter excessive quantities of fluorine in the drinking water may play a role (45). Since fluorine is being used in certain sections of South Africa to prevent dental caries, Steyn feels the problem has become of the utmost practical importance, particularly in obstetrics. He reports, however, that these harmful effects of fluorine can be counteracted by the administration of iodine, whereas they may be intensified by a high calcium content of drinking water (45).

Calcium is an element which has often been associated with the development of goiter (46–48). A high calcium diet apparently results in a greater and more rapid increase in the size of the thyroid glands of those animals maintained on a low-iodine intake (46). Increase in iodine consumption abolishes this effect (46). Perhaps the mode of action of high calcium in the production of goiter is through a combination of this element with iodide, or perhaps the role of iodine deficiency again has not yet been sufficiently ruled out as *the* causative factor. Other elements related to calcium have not been investigated as thoroughly. Silver and barium when in traces in drinking water have been found to bear no relation to the development of goiter (49).

Another element which has received considerable attention as a potentially goitrogenic substance is arsenic. Scott has reported that the incidence of goiter, cretinism, and deaf-mutism in the Styrian Alps, home of the "arsenic-eaters," is considerable (50). Perhaps arsenic through its action of

combining with sulphydryl groups, might impair one of the significant oxidation-reduction systems within the body and thus influence thyroid activity. Hesse has observed that arsenic limits the toxicity and prevents the death of animals "poisoned" by thyroxine and thyroid (51).

Sharpless and Metzger became interested in the goitrogenic effect of arsenic because of the wide use of this substance as an insecticide and antisyphilitic (52). Arsenic fed to animals in nontoxic amounts had a slight but not significant goitrogenic effect. However, with an arsenic concentration of 0.02 per cent in the diet, growth was decreased 50 per cent, iodine concentration of the thyroid diminished and thyroid weight significantly increased. Moreover, the iodine requirement was more than doubled. The administration of iodine was found to reduce the goitrogenic action as well as the toxicity of small amounts of arsenic (52).

GOITROGENIC COMPOUNDS

In addition to those elements which may be either goitrogenic or goitroprophylactic, there are various compounds which have produced definite effects. They are important to consider here since they or related substances may, like vinyl-thio-oxazolidone (1), be among the agents present in foodstuffs, which when given in sufficient amount, can lead to the development of goiter.

The early work which demonstrated the goitrogenic tendencies of these compounds was, at its beginning, incidental. Thus the MacKenzies and McCollum were investigating the effect of sulfaguanidine upon the bacterial synthesis of vitamins in the rat intestine when they noted that enlargement of the thryoid had occurred (53). Similarly, Richter and Clisby, while studying the effect of bitter-tasting substances in rats, found that phenylthiourea would induce goiter (54).

Extensive speculation has revolved around the nature of the active agent responsible for goitrogenic activity in the cabbage family. Marine and his coworkers thought the mustard oils (isothiocyanates), often described as the most characteristic compounds of the genus *Brassica*, to be responsible (55). However, when tested in the rat these were found to be inactive. Marine *et al.* then showed that the cyanides, and particularly acetonitrile, were substantially goitrogenic (55). Many investigators, however, have been unable to confirm these findings (56, 57). One explanation for this variance in results may be found in the work of Bruman and Blomberg (34), which would indicate that the goitrogenicity of acetonitrile is seasonal, depending upon the absence of vitamin D for its action. This or some similar situation may be the key to the differences observed in winter and summer cabbage.

When the goitrogenic properties of thiourea became known, consider-

ation was directed to thiourea or related substances as the antithyroid factor present in foods, especially since it was known that the mustard oils could be converted by ammonia to the thioureas. Allylthiourea was suggested by Kennedy as a possibility (58), for the administration of this substance to experimental animals was found to produce an unusually hyperplastic thyroid. This enlarged thyroid has been described as of the "Basedow" type, and that resulting from benzylthiourea as of the "struma diffusa parenchymatosa" type (2a). If such results are to be expected, then perhaps it is true that a slight change in radical can induce a great change in histopathology.

Thiocyanate, which has been shown to produce thyroid enlargement (59), might also be considered a likely factor. Although the goiter produced by Brassica seeds cannot (7, 17), as in the case of thiocyanate goiter (57, 60), be completely prevented by a sufficient iodine intake, goiter due to edible cabbage (4b) is in this way averted. Thus, thiocyanate, which apparently may be liberated by enzyme hydrolysis from cabbage (15, 61), has not yet been ruled out as a responsible goitrogenic agent in foodstuffs, cabbage in particular.

In 1943 Astwood and his coworkers discovered a modified thiourea compound, thiouracil, which was found to possess powerful metabolism-inhibiting qualities (18). Along with depression of the basal metabolic rate (62–64) and growth retardation (65), the ensuing thyroid hypofunction produced by thiouracil is paralleled by gross as well as microscopic changes in the thyroid gland both in experimental animals and in man. As with promizole administration in rats (66), thyroid hypertrophy, hyperplasia and colloid depletion result (67, 68), and withdrawal of the drug is followed by regression of these findings (67, 68). Furthermore, prolonged use of thiouracil in rats may, as has been recently observed, induce nodular hyperplasia (69) and even varying forms of benign thyroid tumors (70).

By the experimental use of the various goitrogens, not only do their specific effects upon thyroid function become clarified but also thyroid function itself (71). The *first* stage in thyroxine formation (71) lies in the iodide-concentrating mechanism and appears to be a complex ionic effect. Iodide and thiocyanate vie for the same enzyme systems throughout the body (72). Thiocyanate goiter results from interference with the iodide-concentrating mechanism of the thyroid cell (72–75) in what may be described as a mass-action effect of the administered anion (72) and can be prevented by the administration of sufficient iodine (57, 60).

In the *second* stage the concentrated iodide ions are oxidized through tissue redox systems into "free iodine" (71). The free iodine then reacts with tyrosine to form diiodotyrosine. Compounds such as carbon monoxide, the cyanides, azides and sulfides, *in vitro* at least (76), and likewise

the thiourea and thiouracil derivatives (77), may prevent thyroid hormone synthesis by blocking the iodination of hormone precursors. This effect may occur through interference with the cytochrome-cytochrome oxidase and peroxidase enzyme systems (76, 78, 79) or by direct reaction of the antithyroid compound with iodine (71, 77). Aniline derivatives may likewise compete with tyrosine for available iodine (78) or, as in the case of sulfanilamide, exert an inhibitory effect upon the peroxidase system (80), responsible for the liberation of "free iodine" from iodide.

In the *third* stage two diiodotyrosine molecules conjugate to form thyroxine (81) by means of an enzyme system (71, 76). Again, the same substances interfering either directly or through enzyme systems with the liberation of free iodine from iodide or with the reaction of the available iodine with tyrosine, may also block this conjugation. Aniline derivatives, such as *p*-aminobenzoic acid and sulfanilamide, may act through a competitive mechanism in the enzyme system responsible for the conversion of diiodotyrosine to thyroxine (57, 71); the thiouracils may vie with diiodotyrosine for oxidation (71).

There is some indication also that normally tyrosine competes with thyroxine in the tissues and apparently is goitrogenic when the intrinsic source of thyroxine is cut down by feeding thiouracil (71, 82).

In rats fed thiouracil the lowest level of metabolic rate, 40 per cent below normal, has been attained in seventeen days (83). Cretinism itself can be induced in the young of rats receiving methylthiouracil during the period of lactation, and is reversible when the young are separated from their mothers (84). Unlike a parallel case of a cretin born to a hyperthyroid woman receiving this drug (85), cretinism and mortality of the young rats did not ensue when methylthiouracil was administered during gestation alone (84).

The cretin child born of a methylthiouracil-treated hyperthyroid mother lived but one day (85). The infant's thyroid was greatly enlarged and contained no colloid. In another newborn infant under similar prenatal circumstances, exophthalmos and signs of hyperthyroidism were encountered (86). Generally, however, normal children may be expected from hyperthyroid mothers receiving thiouracil medication (87, 88), *provided there is no overdosage*. Overdosage would result in the production of a hypothyroid state with insufficient thyroid hormone to take care of *in utero* requirements (87). Moreover, the thiouracils pass the placenta and thus may directly affect the fetal thyroid. These studies may cast some light, when more facts are available, on the relation of the goitrogens in foods to the appearance of endemic and sporadic cretinism, as well as that of other thyroid disturbances in the newborn.

The action of iodine administered along with the thiouracils is similar

to that when administered along with Brassica seed (7, 17). Its effect is not as extensive as with thiocyanate (57, 60) or with a soybean diet (12–14). The simultaneous administration of iodine along with the thiouracil-like drugs does not entirely prevent their goitrogenic action (89, 90) but, in sufficient amount, can reduce it (90). Diiodotyrosine in high dosage is likewise only partially inhibitory to the action of thiouracil, and like iodine under similar circumstances, accumulates in the nonprotein-bound fraction. On the other hand, thyroxine (18, 90), or even hypophysectomy (18) can prevent the thyroid hypertrophy induced by the thiouracils. These results with thiouracil compounds parallel those with a Brassica-seed diet (17) and thus suggest a close relationship between the thiouracils and the goitrogenic principle of the Brassicae.

Presence of naturally occurring "antithyroid" substances has been sought in animal sources, as well as in plants. One such compound tested is the erythrocyte ergothioneine, belonging to a group of mercaptoimidazoles which exert an "antithyroid" potency (91, 92). Red-cell ergothioneine has been observed to decrease in patients with hyperthyroidism (93); moreover, it has been found to inhibit, *in vitro*, the conversion of acetyldiiodotyrosine to acetylthyroxine (94). The experiments of Astwood and Stanley (95, 96) and of Wilson and McGinty (97) would seem to show that ergothioneine in substantially large doses is inactive in man, rats and monkeys, whereas the experiments of Lawson and Rimington (98) indicate that ergothioneine has an antithyroid activity in rats equivalent to that of thiouracil.

In late 1949, seven years after Astwood's original work on thiouracil, Greer, Ettlinger and Astwood (1) isolated a compound similar to thiouracil, L-5-vinyl-2-thio-oxazolidone from the seeds of rutabaga, turnip, cabbage, kale and rape, and then synthesized this substance in its racemic and optically active forms. All have an equal antithyroid activity, being one fifth as active as thiouracil in the rat and slightly more active than propylthiouracil in man. Presence of vinyl-thio-oxazolidone in Brassica seeds apparently explains the parallelism in mode of action of these seeds and of thiouracil (8, 15, 17, 18, 19, 90).

It was concluded from the amount of thio-oxazolidone isolated and its activity, that this was at least predominantly, if not probably the only, goitrogen contained in these seeds (1). A spectroscopically similar compound was detected in the seeds of Chinese cabbage, Brussels sprouts, broccoli and kohlrabi (1). It could not be demonstrated in cauliflower, mustard or radish. Although the seeds of most Brassicae tested apparently contained thio-oxazolidone or its like, the *edible* portions of none except rutabaga and white turnip yielded a detectable quantity of this compound.

DISCUSSION

Over a period of many years the iodine-deficiency hypothesis has afforded our most satisfying explanation of the pathogenesis of "simple" or endemic goiter. Nevertheless, among those complex physicochemical mechanisms known to ensue in the elaboration and output of the thyroid hormone, admittedly there is more than one factor which can, by its presence or absence, upset the proper balance ordinarily maintained in the normal activity of the thyroid gland. As a consequence, still other "deficiencies" and also "toxic" and even "infectious" agents are constantly being sought in a further effort to explain the still unexplored complexities of the function of the thyroid cells.

The usual medium through which the essential building materials, as well as interfering substances, may enter the organism is by way of the foodstuffs. Evidence now reveals that in addition to iodine lack, certain substances present in foods may contribute to goitrogenesis.

Of the foodstuffs which are most apt to produce experimental goiter in animals, members of the cabbage family (Crucifers) of the genus *Brassica* have been the most widely explored (2, 4, 5, 7, 9). Several other vegetables when fed in *abundance* over a sufficient period of time to experimental animals have also been reported to produce thyroid hyperplasia (2, 10–14). An antithyroid effect of foods has been demonstrated also in man by employing the method of radioactive iodine (I^{131}) uptake (1, 3). There may be some animal species difference in degree of response.

In addition, certain animal products have been suggested as possibly antithyroid (3, 21, 22). Whether this property may be due to the presence of agents similar to those in plant foods is an engaging question, and whether it may reflect the dietary habits of the animals from which the products are derived, is another. Even such foods as animal protein and animal fat, in relative excess (23), have been reported as predisposing factors in an area of endemic goiter. With regard to protein and fat, other conflicting ideas have been expressed (24–27).

The agents in foods responsible for goitrogenesis apparently differ. The effect of some, as of soybean (12–14) and perhaps even of cabbage (4b), is completely counteracted by the administration of iodine, even in small amounts (12–14). On the other hand, the goitrogenic potency of foods such as rutabaga, white turnip and the Brassica seeds (7, 17) can, at most, be only modified by the administration of iodides. In this respect, as well as in the induced histologic changes, the goitrogenic food substances resemble the differing antithyroid compounds now under clinical investigation.

Various compounds have in the past been proposed as those specific agents present in foods which may induce goitrogenesis. These have

included the isothiocyanates (55), the cyanides, particularly acetonitrile (34, 55), and allylthiourea (58). Such compounds, however, have never been established as the responsible antithyroid ingredients. Conversion products of any of these, such as thiocyanate, may still be a possibility in certain foods.

L-5-vinyl-2-thio-oxazolidone is the first of the goitrogenic compounds to be isolated from any food and, moreover, synthesized. It has been demonstrated as the active agent in Brassica seeds and in the edible portions of rutabage and white turnip (1). More extensive investigation of the thiouracils, as well as of the aniline derivatives, may give us a needed further insight into the problem of food goitrogenesis. A study of plant and animal enzymes in relation to these compounds may further aid in the solution of this problem.

Vitamins, at least vitamin A, appear to play some role in thyroid pathology, either by deficiency (28, 29) or excess (30–33). Interacting circumstances may contribute to goitrogenesis, as is suggested for example, by the action of acetonitrile in the absence of vitamin D (34). Certain chemical elements in food besides iodine also have been shown to bear a relation to the development of goiter. These include fluorine (42–45), calcium (46–48), and arsenic (50–52). Two questions which must probably bide their time are: (a) Are there also antigoitrogens in food, and (b) do extrinsic, if not intrinsic, enzymes ever function as goitrogens or antigoitrogens? A deficiency in the intrinsic enzymes essential to normal function of the thyroid cell is at least hypothetically possible and could, in such an instance, act as a goitrogenic stimulus.

SUMMARY

Foods, as well as their various components, have been discussed in relation to the pathogenesis of endemic goiter.

Certain plant foodstuffs, when copiously and persistently consumed have been shown to produce thyroid hyperplasia in experimental animals. Moreover, certain of these foods appear to be antithyroid in man, at least as demonstrated by the inhibition of radioiodine uptake, Particularly, members of the cabbage family have been reported to show such tendencies. The modes of action of the Brassicae (chiefly the seeds) and soybeans differ; the former being partially reversible and the latter, completely reversible, with iodine. This suggests that varying agents may be present in the foods. For that matter, varying agents may be present among the edible Brassicae themselves. Usually, when the responsible goitrogen has not as yet been isolated, one can expect a question as to the role played by iodine deficiency. This is particularly true in those cases in which goitrogenic action of a foodstuff is completely counteracted by iodine.

Various compounds have been held responsible for the goitrogenicity exhibited by certain foods. Convincing proof, however, has been lacking until the recent discovery and isolation of L-5-vinyl-2 thio-oxazolidone from Brassica seeds and then its synthesis. Of the edible portions of the Brassicae, only rutabaga and white turnip thus far have been shown to contain this principle. Other substances suspected as the goitrogenic agents in foods, but not conclusively demonstrated, have included the isothiocyanates, cyanides and thioureas. Thiocyanate and the thiouracil and aniline derivatives, now under extensive investigation with regard to the pathogenesis of thyroid disease, are increasing the range of possibilities.

Certain animal foods have also been discussed as to the presence of antithyroid substances. Elements of the halogen group, arsenic and calcium have been reported under certain circumstances to bear some relation to the origin of goiter. Vitamin A deficiency and hypervitaminosis A have been noted to produce changes in the normal structure of the thyroid gland.

There are also other factors such as infectious processes and undue bodily demands, physiologic and pathologic, which either influence thyroid health directly or indirectly through their effects on the absorption and metabolism of these foodstuffs consumed.

CONCLUSION

With a well-balanced diet, barring deficiency of vital factors such as iodine, and barring *excess* goitrogens, simple goiter could most likely in large measure be avoided.

REFERENCES

1. Greer, M. A.; Ettlinger, M. G., and Astwood, E. B.: Dietary factors in the pathogenesis of simple goiter, *J. Clin. Endocrinol.* **9**: 1069, 1949.
2a. Wagner-Jauregg, T., and Koch, J.: Ernährungsbedingte Schilddrüsenstörungen beim Kaninchen, *Wien. klin. Wchnschr.* **58**: 448, 1946.
2b. Blum, F.: Studien zur Physiologie der Schilddrüse. IV Mitteilung. Kropfnoxe-Basedownoxe, *Endokrinologie* **19**: 19, 1937; Studien zum Kropfproblem. II. Verbreitung von Kropfnoxen im Pflanzenreich und Empfänglichkeit des Tierreichs gegenüber der Schädigung, *Schweiz. med. Wchnschr.* **72**: 1301, 1942; Studien zum Kropfproblem. III Mitteilung. Pflanzliche Kropfnoxen und Gesamtorganismus, *Schweiz. med. Wchnschr.* **73**: 1046, 1943.
3. Greer, M. A., and Astwood, E. B.: The antithyroid effect of certain foods in man as determined with radioactive iodine, *Endocrinology* **43**: 105, 1948.
4a. Chesney, A. M.; Clawson, T. A., and Webster, B.: Endemic goitre in rabbits. I. Incidence and characteristics, *Bull. Johns Hopkins Hosp.* **43**: 261, 1928.
4b. Webster, B., and Chesney, A. M.: Studies in the etiology of simple goiter, *Am. J. Path.* **6**: 275, 1930.
5. Marine, D.; Baumann, E. J., and Cipra, A.: Studies on simple goiter produced by cabbage and other vegetables, *Proc. Soc. Exper. Biol. & Med.* **26**: 822, 1929.
6. McCarrison, R., and Madhava, K. B.: The effect of insanitary conditions on the

thyroid gland and other organs of the body, *Indian J. M. Research* **20:** 637, 697, 1933.

McCarrison, R.; Sankaran, G., and Madhava, K. B.: Effect of an exclusive diet of cabbage on the internal organs of rabbits, *Indian J. M. Research* **20:** 723, 1933.

7. Kennedy, T. H., and Purves, H. D.: Studies on experimental goitre. I. The effect of Brassica seed diets on rats, *Brit. J. Exper. Path.* **22:** 241, 1941.

8a. Griesbach, W. E.: Studies on experimental goitre. II. Changes in the anterior pituitary of the rat, produced by Brassica seed diet, *Brit. J. Exper. Path.* **22:** 245, 1941.

8b. Griesbach, W. E.; Kennedy, T. H., and Purves, H. D.: Studies on experimental goitre. III. The effect of goitrogenic diet on hypophysectomized rats, *Brit. J. Exper. Path.* **22:** 249, 1941.

8c. Griesbach, W. E., and Purves, H. D.: Studies on experimental goitre. V. Pituitary function in relation to goitrogenesis and thyroidectomy, *Brit. J. Exper. Path.* **24:** 174, 1943.

9. Hercus, C. E., and Purves, H. D.: Studies on endemic and experimental goitre, *J. Hyg.* **36:** 182, 1936.

10. Sharpless, G. R.: A new goiter-producing diet for the rat, *Proc. Soc. Exper. Biol. & Med.* **38:** 166, 1938.

11. McCarrison, R.: The goitrogenic action of soya-bean and ground-nut, *Indian J. M. Research* **21:** 179, 1934.

12. Sharpless, G. R.; Pearsons, J., and Prato, G. S.: Production of goiter in rats with raw and with treated soy bean flour, *J. Nutrition* **17:** 545, 1939.

13. Wilgus, H. S., Jr.; Gassner, F. X.; Patton, A. R., and Gustavson, R. G.: The goitrogenicity of soybeans, *J. Nutrition* **22:** 43, 1941.

14. Halverson, A. W.; Zepplin, M., and Hart, E. B.: Relation of iodine to the goitrogenic properties of soybeans, *J. Nutrition* **38:** 115, 1949.

15. Greer, M. A.: Nutrition and goiter, *Physiol. Rev.* **30:** 513, 1950.

16. Marine, D.; Baumann, E. J.; Webster, B., and Cipra, A.: Effect of drying in air on the goiter-producing substance in cabbage, *Proc. Soc. Exper. Biol. & Med.* **27:** 1025, 1930.

17. Purves, H. D.: Studies on experimental goiter. IV. The effect of diiodotyrosine and thyroxine on the goitrogenic action of Brassica seeds, *Brit. J. Exper. Path.* **24:** 171, 1943.

18. Astwood, E. B.; Sullivan, J.; Bissell, A., and Tyslowitz, R.: Action of certain sulfonamides and of thiourea upon the function of the thyroid gland of the rat, *Endocrinology* **32:** 210, 1943.

19. MacKenzie, C. G., and MacKenzie, J. B.: Effect of sulfonamides and thioureas on the thyroid gland and basal metabolism, *Endocrinology* **32:** 185, 1943.

20. Ershoff, B. H.: Protective effects of soybean meal for the immature hyperthyroid rat, *J. Nutrition* **39:** 259, 1949.

21. Hou, H. C.: Thyroid enlargement following liver feeding in rats, *Proc. Soc. Exper. Biol. & Med.* **43:** 753, 1940.

22. Remington, R. E.: Improved growth in rats on iodine deficient diets, *J. Nutrition* **13:** 223, 1937.

23. Bergfeld, W.: Schilddrüse und Ernährung in kropfarmen und kropfreichen Gebieten in Oberbaden, *Ztschr. f.d. ges. exper. Med.* **107:** 106, 1939.

24. Remington, R. E.: Does fat in the diet affect the thyroid, *J. Nutrition* **16:** 417, 1938.

25. Rogers, M. E. C., and Eckstein, H. C.: Goitrogenic effect of dietary fat, *Proc. Soc. Exper. Biol. & Med.* **36:** 738, 1937.
26. Foreign Letters: Postwar goiter in Germany, *J.A.M.A.* **138:** 448, 1948.
27. Jones, H. M.: Self-selected diets in toxic goiter, *J.A.M.A.* **115:** 274, 1940.
28. Coplan, H. M., and Sampson, M. M.: The effects of a deficiency of iodine and vitamin A on the thyroid gland of the albino rat, *J. Nutrition* **9:** 469, 1935.
29. Lipsett, M. B., and Winzler, R. J.: Effects of vitamin A deficiency on thyroid function studied with radioactive iodine, *Endocrinology* **41:** 494, 1947.
30. Sadhu, D. P., and Brody, S.: Excess vitamin A ingestion, thyroid size and energy metabolism, *Am. J. Physiol.* **149:** 400, 1947.
31. Sherwood, T. C.; Toth, L. A., and Carr, K.: Effects of cod-liver oil on basal metabolism and on the thyroid gland, *Endocrinology* **18:** 254, 1934.
32. Sadhu, D. P., and Truscott, B. L.: Hypervitaminosis A and the distribution of body iodine, *Endocrinology* **43:** 120, 1948.
33. Simkins, S.: Use of massive doses of vitamin A in the treatment of hyperthyroidism, *J. Clin. Endocrinol.* **7:** 574, 1947.
34. Bruman, F., and Blomberg, A.: Die Bedeutung des Vitamin D bei der durch Acetonitril erzeugbaren Schilddrüsenhyperplasie, *Ztschr. f.d. ges. exper. Med.* **97:** 229, 1935.
35. Carpenter, M. D., and Sharpless, G. R.: A study of the effect of vitamin B and iodine on the weight, iodine content and structure of the thyroid gland of the rat, *J. Nutrition* **13:** 235, 1937.
36. Harris, P. L., and Remington, R. E.: The effect of yeast and of thiamin on the production of low iodine goiter, *J. Nutrition* **17:** 31, 1939.
37. Marine, D.; Baumann, E. J., and Rosen, S. H.: Effect of ascorbic acid on thyroid and suprarenals of guinea pigs, *Proc. Soc. Exper. Biol. & Med.* **31:** 870, 1934.
38. Spence, A. W., and Scowen, E. F.: The effect of ascorbic acid on experimental goitre, *Biochem. J.* **29:** 562, 1935.
39. McCarrison, R.: The pathogenesis of deficiency disease. X. The effects of some food deficiencies and excesses on the thyroid gland, *Indian J. M. Research* **7:** 633, 1920.
40. Andreev, F.: About the etiology of mass goiter, *Voen.-med. J.* (*Moskva*) **2:** 351, 1931.
41. Emelyanova, E. N.: New data on action of bromine salts on thyroid gland, *Am. Rev. Soviet Med.*, N. Y. **4:** 421, 1947.
42. Williams, R. H.; Jaffe, H., and Soloman, B.: Effect of halides on the antithyroid action of thiouracil and propylthiouracil, *Am. J. M. Sc.* **219:** 1, 1950.
43. Wilson, D. C.: Fluorine in the aetiology of endemic goitre, *Lancet* **1:** 211, 1941.
44. May, W.: Antagonismus zwischen Jod und Fluor im Organismus, *Klin. Wchnschr.* **14:** 790, 1935.
45. Steyn, D. G.: Fluorine and endemic goiter, *S. African Med. J.*, Cape Town **22:** 525, 1948.
46. Thompson, J.: Influence of the intake of calcium on the thyroid gland of the albino rat, *Arch. Path.* **16:** 211, 1933.
47. Wahlberg, J.: Deficiency of iodine and excess of calcium within an endemic goiter area, *Tr. Third Internat. Goiter Conference and Am. A. Study Goiter*, 1938, p. 71.
48. Hellwig, C. A.: Experimental goiter due to calcium, *Arch. Surg.* **40:** 98, 1940.
49. Boissevain, C. H., and Drea, W. F.: Relation between the occurrence of endemic

goiter and the presence of traces of silver and barium in the drinking water, *Endocrinology* **20:** 686, 1936.

50. Scott, M.: The possible role of arsenic in the etiology of goiter, cretinism and endemic deaf mutism, *Tr. Third Internat. Goiter Conference and Am. A. Study Goiter*, 1938, p. 34.
51. Hesse, E.: Die Entgiftung der Schilddrüsenhormons durch Metalle und natürliche Quellen, *Klin. Wchnschr.* **12:** 1060, 1933.
52. Sharpless, G. R., and Metzger, M.: Arsenic and goiter, *J. Nutrition* **21:** 341, 1941.
53. MacKenzie, J. B.; MacKenzie, C. G., and McCollum, E. V.: Effect of sulfanilylguanidine on the thyroid of the rat, *Science* **94:** 518, 1941.
54. Richter, C. P., and Clisby, K. H.: Graying of hair produced by ingestion of phenylthiocarbamide, *Proc. Soc. Exper. Biol. & Med.* **48:** 684, 1941. Toxic effects of bitter-tasting phenylthiocarbamide, *Arch. Path.* **33:** 46, 1942.
55. Marine, D.; Bauman, E. J.; Spence, A. W., and Cipra, A.: Further studies on etiology of goiter with particular reference to the action of cyanides, *Proc. Soc. Exper. Biol. & Med.* **29:** 772, 1931.
56. Spence, A. W.: The effect of the administration of cyanides on the thyroid gland of chickens, *J. Pharmacol. & Exper. Therap.* **48:** 327, 1933.
57. Astwood, E. B.: The chemical nature of compounds which inhibit the function of the thyroid gland, *J. Pharmacol & Exper. Therap.* **78:** 79, 1943.
58. Kennedy, T. H.: Thioureas as goitrogenic substances, *Nature* **150:** 233, 1942.
59. Barker, M. H.: The blood cyanates in the treatment of hypertension, *J.A.M.A.* **106:** 762, 1936.
60. Salter, W. T.; Cortell, R. E., and McKay, E. A.: Goitrogenic agents and thyroidal iodine: their pharmacodynamic interplay upon thyroid function, *J. Pharmacol. & Exper. Therap.* **85:** 310, 1945.
61. McCarrison, R.: Food and goitre, *Brit. M. J.* **2:** 671, 1933. The interaction of food and sanitary conditions in the causation and prevention of thyroid disease, *Indian J. M. Research* **20:** 957, 1933.
62. Barker, S. B.: Effects of thyrotrophin on metabolism of thiouracil-treated rats, *Endocrinology* **37:** 230, 1945.
63. Meyer, A. E., and Ransom, G. W.: The metabolism of rats after thyroidectomy or during thiouracil treatment, and the effect of thyroid feeding, *Endocrinology* **36:** 259, 1945.
64. Reineke, E. P.; Mixner, J. P., and Turner, C. W.: Effect of graded doses of thyroxine on metabolism and thyroid weight of rats treated with thiouracil, *Endocrinology* **36:** 64, 1945.
65. Goldsmith, E. D.; Gordon, A. S., and Charipper, H. A.: An analysis of the effects of continued thiourea treatment in pregnancy and on the development of the offspring in the rat, *Am. J. Obst. & Gynec.* **49:** 197, 1945.
66. Higgins, G. M.: A study of the goitrogen, promizole, with reference to the thyroid, metabolism and the blood, *Am. J. M. Sc.* **210:** 347, 1945.
67. Reveno, W. S.: Effect of thiouracil on human tissues, *J. Clin. Endocrinol.* **5:** 403, 1945.
68. Shirer, J. W., and Cohen, M.: The effects of thiouracil on the thyroid gland, *Ann. Int. Med.* **23:** 790, 1945.
69. Laqueur, G. L.: Nodular hyperplasia of thyroid glands induced by thiouracil, *Cancer Research* **9:** 247, 1949.
70. Money, W. L., and Rawson, R. W.: Experimental production of thyroid tumors in rat exposed to prolonged treatment with thiouracil, *Cancer* **3:** 321, 1950.

71. SADHU, D. P.: Physiological mechanism of experimental goitrogenesis, *Am. J. Physiol.* **152:** 150, 1948.
72. BAUMANN, E. J., and METZGER, N.: Action of thiocyanates in producing goiter, *Fed. Proc.* **6:** 237, 1947.
73. VANDERLAAN, W. P., and BISSELL, A.: The influence of selected goitrogenic compounds on the thyroid gland of the chick, *Endocrinology* **38:** 308, 1946. Effects of propylthiouracil and of potassium thiocyanate on the uptake of iodine by the thyroid gland of the rat, *Endocrinology* **39:** 157, 1946.
74. VANDERLAAN, J. E., and VANDERLAAN, W. P.: The iodide concentrating mechanism of the rat thyroid and its inhibition by thiocyanate, *Endocrinology* **40:** 403, 1947.
75. WOLFF, J.; CHAIKOFF, I. L.; TAUROG, A., and RUBIN, L.: The disturbance in iodine metabolism produced by thiocyanate. The mechanism of its goitrogenic action with radioactive iodine as indicator, *Endocrinology* **39:** 140, 1946.
76. SCHACHNER, H.; FRANKLIN, A. L., and CHAIKOFF, I. L.: The effect of cytochrome oxidase inhibitors on the formation in vitro of thyroxine and diiodotyrosine by thyroid tissue with radioactive iodine as indicator, *J. Biol. Chem.* **151:** 191, 1943.
77. MILLER, W. H.; ROBLIN, R. O., JR., and ASTWOOD, E. B.: Studies in chemotherapy. XI. Oxidation of 2-thiouracil and related compounds by iodine, *J. Am. Chem. Soc.* **67:** 2201, 1945.
78. DEROBERTIS, E., and GRASSO, R.: Peroxidase activity of the thyroid gland under normal and experimental conditions, *Endocrinology* **38:** 137, 1946.
79. SUMNER, J. B., and SOMERS, G. F.: Chemistry and Methods of Enzymes. New York, Academic Press, Inc., 1943.
80. LIPMANN, F.: The oxidation of *p*-aminobenzoic acid catalyzed by peroxidase and its inhibition by sulfanilamide, *J. Biol. Chem.* **139:** 977, 1941.
81. VON MUTZENBECHER, P.: Über die Bildung von Thyroxin aus Dijodtyrosin, *Zeitr. physiol. Chem.* **261:** 253, 1939.
82. JOHNSON, T. B., and TEWKESBURY, L. B., JR.: The oxidation of 3, 5-diiodotyrosine to thyroxine, *Proc. Nat. Acad. Sc.* **28:** 73, 1942.
83. SADHU, D. P., and BRODY, S.: Thiouracil, basal metabolism and specific dynamic action, *Am. J. Physiol.* **151:** 130, 1947.
84. BICKENBACH, W.; HESS, M., and LOESER, A.: Über die Anwendung von Methylthiouracil während der Schwangerschaft und der Stillzeit, *Deutsche med. Wchnschr.* **73:** 640, 1948.
85. HONE, G. M., and MAGAREY, I.: Cretinism associated with methyl thiouracil therapy, *Med. J. Australia* **2:** 524, 1948.
86. FRISK, A. R., and JOSEFSSON, E.: Thiouracil derivatives in pregnancy, *Acta med. Scandinav.* **128:** Supp. 196, 85, 1947.
87. CAREN, R.: The goitrogens in thyrotoxicosis complicating pregnancy. A case report, *J. Clin. Endocrinol.* **9:** 903, 1949.
88. WILLIAMS, R. H.: Thiouracil treatment of thyrotoxicosis. I. The results of prolonged treatment, *J. Clin. Endocrinol.* **6:** 1, 1946.
89. RIKER, W. P., and WESCOE, W. C.: The pharmacology and therapeutic applications of antithyroid compounds, *Am. J. M. Sc.* **210:** 668, 1945.
90. MCGINTY, D. A.: Iodine absorption and utilization under the influence of certain goitrogens, *Ann. New York Acad. Sc.* **50:** 403, 1949.
91. ASTWOOD, E. B.; BISSELL, A., and HUGHES, A. M.: Further studies on the chemical nature of compounds which inhibit the function of the thyroid gland, *Endocrinology* **37:** 456, 1945.

92. JACKMAN, M.; KLENK, M.; FISHBURN, B.; TULLAR, B. F., and ARCHER, S.: The preparation of some substituted thiohydantoins and thioimidazoles, *J. Am. Chem. Soc.* **70:** 2884, 1948.
93. LATNER, A. L., and MOWBRAY, R.: Blood ergothioneine values in normal individuals and thyrotoxicosis, *Biochem. J.* **42:** 35, 1948.
94. PITT-RIVERS, R.: The action of antithyroid substances on the formation *in vitro* of acetylthyroxine from acetyldiiodotyrosine, *Biochim. et biophys. acta* **2:** 311, 1948.
95. ASTWOOD, E. B., and STANLEY, M. M.: Antithyroid activity of ergothioneine in man, *Lancet* **2:** 905, 1947.
96. STANLEY, M. M., and ASTWOOD, E. B.: Determination of the relative activities of antithyroid compounds in man using radioactive iodine, *Endocrinology* **41:** 66, 1947.
97. WILSON, M. L., and MCGINTY, D. A.: Antithyroid activity of ergothioneine, *Am. J. Physiol.* **156:** 377, 1949.
98. LAWSON, A., and RIMINGTON, C.: Antithyroid activity of ergothioneine; normal component of blood, *Loncet* **1:** 586, 1947.

EXCRETION OF STEROIDAL SUBSTANCES BY THE ADRENAL CORTEX IN VARIOUS DISEASES*

KAMBI SHADAKSHARAPPA, M.Sc.†, N. O. CALLOWAY, PH.D., M.D.,‡ R. H. KYLE, M.D.§ AND R. W. KEETON, M.D.

From the Department of Medicine, University of Illinois College of Medicine, Chicago, Illinois

FOR many years it has been suspected that the adrenal glands play an important role in the pathogenesis of diseases not etiologically related to these glands. Further, it has been suggested with increasing frequency that the adrenal cortex plays a vital role in known and unknown homeostatic mechanisms which have sufficient lability to maintain physiologic function but which, when deranged, may ameliorate, complicate or actually cause disease itself.

Since it was not possible in this study to evaluate the adrenal glands completely by all of the known tests, we selected the determination of urinary phosphomolybdate-reducing substances, which have at times been designated as "neutral reducing lipids," and 17-ketosteroids as having significance indicative of the physiologic state of the adrenal cortex. These phosphomolybdate-reducing substances will be referred to in the remainder of the paper as Ph.S.

Other groups have studied several aspects of this problem using different methods for cortical steroid determination (1–5).

This is a preliminary report on the activity of the adrenal cortex in disease. We are in the process of examining some of the diseases in more detail, and these results will be set forth in future publications. The inadequate tests, the complexity of the responses of the adreno-endocrine system, and the myriad of poorly understood or unrecognized labile physiologic mechanisms render any report at this time only a series of observations which may serve as a springboard for more fruitful, orderly, and specific investigation concerning the pathogenesis of various diseases as related to the adrenals.

Received for publication October 25, 1950.

* This material is essentially an abstract of a thesis submitted in partial fulfillment of the requirements for the Degree of Master of Science in Internal Medicine by Kambi S. Shadaksharappa to the Graduate Faculty of the College of Medicine, University of Illinois, Chicago, Illinois.

† Present address: Lalita 12, Shankarpuram, Bangalore 4, India.

‡ Presently Major, M.C., Percy Jones Army Hospital, Battle Creek, Michigan.

§ Junior Fellow of the National Heart Institute of the United States Public Health Service.

METHODS OF STUDY

Patients from the wards of the hospital were used. They were kept under constant conditions of activity, diet and medication during this study.

Determinations were carried out on 24-hour urine specimens. Although it has been shown that the output of 17-ketosteroids in a 24-hour period is only approximately constant from day to day in a given subject (6, 7, 8), the fluctuations are not marked (9).

The method used for the determination of Ph.S. in urine was that of Heard and Sobel (10). The 17-ketosteroids were determined by the method of Robbie and Gibson (11). The epinephrine test of adrenal response was that of Recant (12).

Normal values

In 6 males whose average age was 30 years, the normal average value for 24-hour urinary excretion of Ph.S. was 1.7 mg. (range, 1.25 to 2 mg.), and of 17-ketosteroids 11.5 mg. (range 8 to 14.2 mg.). In 5 females whose average age was 27 years, the Ph.S. value was 1.3 mg. (range, 1.2 to 1.6 mg.) and 17-ketosteroids, 8 mg. (range 6.5 to 10 mg.). All estimations were made by the same workers.

RESULTS AND DISCUSSION

Hypertensive states

It has been shown (13, 14, 15) that bilateral adrenalectomy prevents the development of hypertension which is produced by the constriction of renal arteries and that the hypertension so induced is not maintained if the adrenals are removed. It is also well known that impaired adrenal function is clinically associated with hypotension. Goldblatt (16) thinks that of the endocrine organs the only one that may possibly play a significant role, if only a secondary part, is the adrenal gland.

Excessive administration of desoxycorticosterone acetate along with salt may cause hypertension in Addison's disease (17, 18, 19), in adrenalectomized animals (20) and in normal and hypertensive animals (20, 21, 22). However, even very large doses of adrenal cortical extract have failed to produce hypertension. The production by Selye (23) and his co-workers of nephrosclerosis, hypertension, periarteritis nodosa, and rheumatic lesions by the administration of desoxycorticosterone acetate (24) or 17-hydroxy-11-desoxycorticosterone (25) and sodium chloride in several species of animals has been the best experimental attempt to relate the adrenal cortex to hypertension and the kidney.

Small adenomas of the adrenal cortex have been described in about 1.45 per cent of all autopsies, and patients showing these changes frequently exhibit hypertension in life (26). Hyperplasia of the adrenal cortex

associated with hypertension has been described (27). This has been questioned by others (28). Dempsey (29) has stated that hypertension is not necessarily accompanied by any specific alteration in the structure of the adrenal cortex and that the size of the adrenal cortex is not altered by the existing hypertensive state.

In our series of 24 cases of hypertensive diseases (Table 1), the excretion of 17-ketosteroids was decreased. The excretion of Ph.S. was increased

TABLE 1. URINARY 17-KETOSTEROIDS AND PH.S. IN HYPERTENSIVE PATIENTS

Case No.	Patient, initials	Sex	Age	Disease	Urine albumin	B.P. (mm. Hg)	Ocular fundi	Ph.S., mg./24 hrs.	17-KS, mg./24 hrs.	Eosinophils per cu. mm.
1	W.P.	F	22	Essential hypertension	trace	240/154	Gr. II	1.8	5.0	200
2	M.F.	F	41	Essential hypertension	+	230/130	Gr. I	2.6	5.2	142
3	S.H.	F	30	Essential hypertension	nil	190/120	Gr. I	2.1	5.5	186
4	M.W.	F	40	Essential hypertension	nil	190/130	Gr. I	2.2	5.8	133
5	T.B.	F	55	Essential hypertension	—	262/150	Gr. II	1.2	4.2	—
6	G.G.	M	33	Essential hypertension	—	220/130	Gr. II	2.2	6.5	133
7	M.M.	F	42	Hypertension, hirsutism, obesity	trace	182/104	Gr. I	3.2	14.0§	120
8	M.C.	F	43	Hypertension, hirsutism, obesity	trace	184/112	Gr. II	2.9	5.0	103
9	G.C.	M	27	Ess. hypertension & rheumatic heart disease	nil	190/40	Gr. II	3.2	5.5	300
10	M.B.	F	64	Hypertension & arteriosclerosis	trace	185/110	Gr. I	1.3	3.5	—
11	M.H.	F	52	Ess. hypertension & diabetes	nil	228/130	Gr. II	2.2	4.2	180
12	B.G.	F	66	Ess. hypertension & diabetes	—	180/110	Gr. IV	4.5	3.2	20
13	A.H.	F	49	Ess. hypertension & diabetes	trace	240/140	Gr. III	2.4	3.2	200
14	H.R.	M	28	Ess. hypertension & obesity	+	160/100	normal	1.6	5.8	260
15	J.F.	M	45	Ess. hypertension with early failure	+++	185/140	Gr. III	3.4	4.8	—
16	T.J.	F	62	Ess. hypertension with early failure	+++	200/110	Gr. III	2.7	3.0	—
17	G.N.	M	62	Ess. hypertension with early failure	trace	170/90	Gr. I	2.4	5.8	—
18	E.T.	M	58	Ess. hypertension with early failure	nil	170/110	Gr. I	2.7	3.45	50
19	E.M.	M	23	Diabetes & hypertension (K.W.S.)‖	+++	170/100	Gr. IV	3.4	3.5	178
20	F.S.*	F	48	Diabetes & hypertension (K.W.S.)‖	+++	266/142	Gr IV	3.65	2.8	5
21	G.L.	M	26	Diabetes & hypertension (K.W.S.)‖	+++	210/120	Gr. IV	3.2	4.0	—
22	R.R.	M	19	Renal rickets, hypertension, uremia	+++	150/105	Gr. II	2.8	3.2	72
23	V.F.†	F	48	Hypertension, nephrosclerosis, uremia	+++	260/170	Gr. III	4.0	2.5	6
24	A.F.‡	M	48	Chr. pyelonephritis & uremia	++++	185/105	Gr. IV	4.8	2.0	5
Average for males								*3.0*	*4.5*	
Average for females								*2.6*	*4.1*	

* Deceased. Adrenal cortex normal in size and shape; microscopic examination showed hyperplasia.
† Deceased. Adrenal cortex normal in size and shape; microscopic examination showed normal structure.
‡ Deceased. Adrenal cortex normal in size and shape; microscopic examination showed a few scattered adenomas.
§ Not included in averages.
‖ K.W.S.—Kimmelstiel-Wilson syndrome.

in 20 and more so in patients with kidney damage or early ventricular failure. In the 3 fatalities (Table 1) the increase in excretion of Ph.S. material was significant, and in view of the very low eosinophil count a considerable portion of the increase in value of Ph.S. may have been the result of excess glucocorticoids. This method of estimation is only semiquantitative, using Ph.S. to measure the glucocorticoids, mineral corticoids, part

of the androgens, and perhaps unknown compounds. The chief endocrine offenders (22, 25) in the causation of experimental hypertension are 11-desoxycorticosterone acetate (which is a mineral corticoid) and 17-hydroxy-11-desoxycorticosterone. At this time there is no satisfactory method to estimate only the mineral corticoids in blood and urine. Further, the increased corticotropin production during stress has not been measured directly. The amount of desoxycorticosterone acetate extracted from adrenal cortex is small and to date the work of Reichstein in isolating desoxycorticosterone from the adrenal cortex has not been reproduced.

In our cases of essential hypertension (Table 1) the average value for

TABLE 2. URINARY 17-KETOSTEROIDS AND PH.S. IN RHEUMATIC FEVER

Patient				Blood sed. rate, mm./hr.	B.P. (mm. Hg)	Ph.S., mg./24 hrs.	17-KS, mg./24 hrs.	Eosinophils per cu. mm.
Case No.	Initials	Sex	Age					
1	B.H.	F	15	22	145/80	2.85	4.8	50
2	A.L.	F	12	21	105/65	2.2	3.45	272
3	A.B.	F	6	42	80/50	2.0	1.5	216
4	E.D.	F	12	48	125/50	2.6	2.0	666
5	J.L.	F	12½	34	120/80	2.5	2.9	486
6	M.F.	F	12	20	95/55	2.3	1.8	220
					Average	*2.4*	*2.8*	

the Ph.S. was 1.9 mg. per twenty-four hours which is a questionably significant elevation and confirms the findings of others (3, 5). In fact, 3 of the 7 uncomplicated cases fell in the normal range.

In those patients in whom hirsutism and obesity were present without sufficient clinical evidence to suspect Cushing's syndrome, there was slight but definite elevation of the Ph.S. and in 1 subject, No. 7, there was significant elevation of the 17-ketosteroids above the values found for similar patients. These patients are being studied further.

Rheumatic fever

In all the cases of rheumatic fever in our series (Table 2), there was increased excretion of Ph.S., whereas 17-ketosteroid excretion was decreased. Though rheumatic lesions are produced by giving desoxycorticosterone acetate and salt to unilaterally nephrectomized animals, such lesions have not been reported during administration of desoxycorticosterone acetate to humans. Here the increased excretion of Ph.S. probably is a

result of stress and there is no evidence that increased corticoids have produced the disease. One should rather look for the cause of the stress in the rheumatic fever.

Cirrhosis of the liver

Numerous observations in animals (30), as well as in human subjects (31, 32), have shown that the liver plays a dominant role in metabolism of estrogens, though its exact nature is not well known. In males with cirrhosis of the liver the appearance of gynecomastia and testicular atrophy is attributed to an increase in unconjugated urinary estrogens (32). There is an increase in urinary excretion of estrogens and a decreased excretion of 17-ketosteroids in patients with liver damage due to infectious hepatitis (33). Evidence has been presented to indicate that liver insufficiency interferes with cholesterol metabolism of the adrenals in animals (34). The fundamental structure of steroids, sex hormones and adrenal cortex hormones is the cyclopentenophenanthrene ring system and hence it is likely that there is considerable upset in the metabolism of steroid hormones, which probably accounts for the high Ph.S. value. In our cases of cirrhosis of the liver, the excretion of 17-ketosteroids was decreased and that of Ph.S. was increased (Table 3). It is significant that there was a high excretion of Ph.S. in the normotensive patients in this group.

TABLE 3. URINARY 17-KETOSTEROIDS AND PH.S. IN CIRRHOSIS OF THE LIVER

Patient				B.P. (mm. Hg)	Ph.S., mg./24 hrs.	17-KS, mg./24 hrs.	Eosinophils per cu. mm.
Case No.	Initials	Age	Sex				
1	L.F.	58	M	116/68	4.5	1.6	100
2	T.W.*	58	M	118/62	3.8	0.75	140
3	K.K.	54	M	112/72	3.3	1.8	343
4	A.M.	32	M	98/56	4.3	1.2	66
5	P.W.	52	M	108/62	5.7	2.2	121
6	B.F.	60	M	128/70	3.8	1.4	143
Average					*4.2*	*1.5*	

* Deceased. Both kidneys and adrenal cortex were normal, macroscopically and microscopically.

Thyroid disease

Athyroid adrenalectomized animals survive longer than adrenalectomized animals (35). Administration of thiouracil leads to a reduction in metabolic activity and to adrenal cortical atrophy (36). Administration

of thyroxine to animals results in an increase of weight of the adrenals (37). The basal metabolism is reduced in Addison's disease and giving thyroxine to a patient with Addison's disease has a deleterious effect. In our 2 cases of myxedema there was a definite decrease in excretion of both Ph.S. and 17-ketosteroids, confirming the findings of others (2, 3, 11, 38), whereas in 4 out of 5 cases of hyperthyroidism there was an increased excretion of Ph.S. but 17-ketosteroids were decreased. Using different methods, some investigators (1) found normal corticoids in a hyperthyroid patient, whereas others (3) in 9 cases found 1 elevated, 1 normal and 7 subnormal levels of formaldehydogenic steroids. In hyperthyroidism the patient is under considerable stress, with consequent increase in the requirement of adrenal cortical hormone; the reverse is the case in myxedema. In 1 patient with severe hyperthyroidism the Ph.S. excretion was low. Perhaps there was increased utilization or an exhaustion of the adrenals. In dogs, adrenal cortical extract appears to counteract the deleterious effect of thyroxine on nitrogen balance (39). It is likely that functional change in tissue cells leads to an increased hormone utilization in stress and the increased production of Ph.S. is a part of homeostatic adjustment. This suggests a definite relationship between adrenal cortex and thyroid (Table 4).

TABLE 4. URINARY 17-KETOSTEROIDS AND PH.S. IN HYPOTHYROIDISM AND IN HYPERTHYROIDISM

Patient				B.M.R., %	Ph.S., mg./24 hrs.	17-KS, mg./24 hrs.
Case No.	Initials	Sex	Age			
1	D.T.	F	15	−30	0.5	1.65
2	A.M.	F	11	−25	0.45	1.8
3	J.L.	F	63	+47	3.0	3.5
4	M.L.	F	49	+59	2.65	4.5
5	B.R.	F	12	+50	2.16	3.0
6	M.F.	F	13	+32	2.4	3.6
7	L.K.	F	34	+81*	0.95	5.0
Hypothyroidism—average					*0.5*	*1.7*
Hyperthyroidism—average					*2.3*	*4.0*

* In this 1 case of hyperthyroidism, both the urinary Ph.S. and 17-ketosteroids were decreased.

Diabetes

In patients with uncomplicated diabetes (Table 5) the urinary excretion of 17-ketosteroids was decreased, whereas the excretion of urinary Ph.S.

was within normal limits. Others (1) have found normal values for both 17-ketosteroids and glycogenic corticoids. The diabetes in these patients was fairly well controlled. In view of the role the adrenal cortex plays in carbohydrate metabolism, more information may be obtained by studying untreated diabetics (40, 41).

TABLE 5. URINARY 17-KETOSTEROIDS AND PH.S. IN DIABETES MELLITUS

Patient				Ph.S., mg./24 hrs.	17-KS, mg./24 hrs.
Case No.	Initials	Sex	Age		
1	M.B.	F	17	1.55	4.50
2	D.G.	M	17	1.46	5.00
3	G.M.	M	15	1.40	4.25
4	M.H.	F	18	1.70	5.50
5	C.S.	M	24	1.90	6.20
Average				*1.60*	*5.10*

Leukemia

The recent evidence indicates that functional activity of lymphoid tissue is intimately related to activity of the adrenal cortex. In view of the possible involvement of the adrenal cortex in leukemia, the excretion of urinary Ph.S. and 17-ketosteroids was determined in 2 patients with chronic lymphatic leukemia. A decrease was found in the excretion of 17-ketosteroids (1.8 mg. per twenty-four hours in Patient L.D. and 3.8 mg. in J.F.) and an increase in the excretion of urinary Ph.S. (3.6 mg. per twenty-four hours in L.D. and 2.6 mg. in J.F.). It has been shown that following administration of ACTH there is a fall in lymphocytes to 50 per cent of that in normals. With this in mind, a dose of 25 mg. of ACTH was given to a patient (L.D.). There was neither a significant fall in lymphocytes nor a significant change in the excretion of 17-ketosteroids and Ph.S. In Patient J.F., a total of 50 mg. of ACTH given in divided doses was not accompanied by a fall in the lymphocytic count, but there was considerable increase in the urinary excretion of 17-ketosteroids and of Ph.S. as well as a significant fall in eosinophils.

Anorexia nervosa

Venning and Browne (1) found low 17-ketosteroids and normal to slightly subnormal glycogenic corticoids in anorexia nervosa. In malnutrition, the pituitary passes into a stage of functional hypoactivity which results secondarily in hypofunction of other endocrine glands. Selye (42) has noted

less secretion of pituitary gonadotropins during malnutrition.

In our case of anorexia nervosa the excretion of urinary 17-ketosteroids (1.8 mg. per twenty-four hrs.) as well as Ph.S. (0.5 mg. per twenty-four hrs.) was reduced, indicating the hypofunctional state of adrenal cortex. The poor response to epinephrine also suggests that there was hypofunction of the pituitary or adrenal cortex. The normal response to the 25-mg. ACTH test of Thorn *et al.* (43) indicates that function of the adrenal cortex was normal but that it was not receiving adequate stimulation from the pituitary gland. The patient subsequently showed a steady improvement with tube feeding and medical management.

Postoperative patients

In 3 patients, following surgical procedures, there was no significant rise in the urinary 17-ketosteroids but there was an increase in the excretion of urinary Ph.S., confirming the observations of others (2). After the third postoperative day there was a decrease in 17-ketosteroid excretion but excretion of urinary Ph.S. remained elevated for some time. This suggests that excretion of Ph.S. is a more sensitive index of adrenal cortical activity. However, the test does not differentiate between mineral corticoids, glucocorticoids and androgens, and leaves one in doubt as to which class of substances is increased.

Cryptorchidism

In a cryptorchid patient, following injection of anterior pituitary-like substance (A.P.L.), there was no significant rise in the excretion of 17-ketosteroids or of Ph.S., but on giving 25 mg. of ACTH there was a significant increase in both 17-ketosteroids and Ph.S. This suggests that in this patient the principal source of 17-ketosteroids was the adrenal cortex.

Adrenal and gonadal lesions

In 2 cases of Cushing's syndrome, the excretion of urinary 17-ketosteroids was only slightly elevated but the excretion of Ph.S. was high (Table 6, Cases 1 and 2), confirming the findings of others (1, 2, 3). The low eosinophil count suggests the excess production of glucocorticoids. Both patients were hypertensive. In the cases of pseudohermaphrodism and of sexual precocity (Table 6, Cases 3 to 7) the 17-ketosteroids were elevated but the Ph.S. values were only slightly elevated, confirming in general the observations of others (1, 2, 3). Heard, Sobel and Venning (44) have reported in 4 normal children aged $2\frac{1}{2}$ to 7 years, excretions of Ph.S. ranging from 0.32 to 0.79 mg. per twenty-four hours. The elevation of 17-ketosteroids can be best appreciated by comparison with values from normal children of similar age (45) which ranged from 0.15 to 1.4 mg. In

TABLE 6. URINARY 17-KETOSTEROIDS AND PH.S. IN CUSHING'S AND ADRENOGENITAL SYNDROMES

Patient				Ph.S., mg./24 hrs.	17-KS, mg./24 hrs.	Eosinophils per cu. mm.	Diagnosis
Case No.	Initials	Sex	Age				
1	M.T.	F	32	8.5	18.0	19	Cushing's syndrome
2	C.V.	F	34	4.8	17.0	26	Cushing's syndrome
3	W.T.	F	5	1.0	6.5		Sexual precocity
4	J.T.	M	4½	0.75	6.0	372	Sexual precocity
5	D.T.	F	9	0.9	9.0	−746	Pseudohermaphrodism
						−130	
6	N.T.	F	2	0.43	4.03		Pseudohermaphrodism
7	W.F.	F	6	0.75	1.0	320	Pseudohermaphrodism
8	A.B.	F	17	5.8	88.0	189	Virilism
			After surgery	2.0	6.8		

a case of virilism (Table 6, Case 8) due to adenoma of the adrenal, both the Ph.S. and 17-ketosteroid excretions were increased but they returned to normal following surgery.

CONCLUSIONS

1. The excretion of urinary phosphomolybdate-reducing substances (Ph.S.) and of 17-ketosteroids has been studied in patients with various diseases.

2. The observations of previous workers that there is a low urinary excretion of 17-ketosteroids in hypertension, cirrhosis of the liver, rheumatic diseases, diabetes, anorexia nervosa, chronic lymphatic leukemia, myxedema and hyperthyroidism, were confirmed.

3. The urinary excretion of Ph.S. was increased in hypertension, rheumatic diseases, cirrhosis of the liver, hyperthyroidism and chronic lymphatic leukemia.

4. The values for 17-ketosteroids and Ph.S. did not always run parallel. Ph.S. excretion appears to be a more sensitive index of adrenal cortical activity, although it does not show to what extent the mineral corticoids, glucocorticoids and the androgens individually are stimulated by stress.

5. In Cushing's syndrome, the values for urinary Ph.S. were high and those for 17-ketosteroids were within the upper limits of normal, or slightly elevated. In the adrenogenital syndrome the 17-ketosteroid excretion was increased, whereas Ph.S. excretion was only slightly elevated.

6. There was increased Ph.S. or corticoid excretion in hypertension and

in rheumatic disease, but with the available evidence it is difficult to say that the increased corticoids were causally related to these two diseases which are included under Selye's so-called "diseases of adaptation."

7. Following injection of anterior pituitary-like substance into a cryptorchid patient there was no significant rise in urinary 17-ketosteroids or in Ph.S., but there was a significant rise in both following ACTH administration.

8. A low 17-ketosteroid value is found in a variety of diseases and may reflect the state of general nutrition of the patients. When there is a significant rise in 17-ketosteroids it is reasonable to suspect changes in the activity of the adrenal cortex.

9. Administration of 50 mg. of ACTH failed to show any change in the lymphocytic count in a case of chronic lymphatic leukemia, but caused a drop in the eosinophils.

Acknowledgment

The authors wish to express appreciation to Dr. H. G. Poncher and Dr. I. P. Bronstein of the Department of Pediatrics of the University of Illinois for making available clinical material for part of the study.

REFERENCES

1. Venning, E. H., and Browne, J. S. L.: Excretion of glycogenic corticoids and of 17-ketosteroids in various endocrine and other disorders, *J. Clin. Endocrinol.* **7**: 79, 1947.
2. Talbot, N. B.; Albright, F.; Saltzman, A. H.; Zygmuntowicz, A., and Wixom, R.: The excretion of 11-oxycorticosteroid-like substances by normal and abnormal subjects, *J. Clin. Endocrinol.* **7**: 331, 1947.
3. Daughaday, W. H.; Jaffe, H., and Williams, R.: Adrenal cortical hormone excretion in endocrine and nonendocrine disease as measured by chemical assay, *J. Clin. Endocrinol.* **8**: 244, 1948.
4. Daughaday, W. H.; Jaffe, H., and Williams, R.: Chemical assay for "cortin," *J. Clin. Endocrinol.* **8**: 166, 1948.
5. Tobian, L., Jr.: Cortical steroid excretion in edema of pregnancy, pre-eclampsia, and essential hypertension, *J. Clin. Endocrinol.* **9**: 319, 1949.
6. Bachman, C.; Leekley, D., and Winter, B.: Day and night excretion of pregnandiol glucuronidate and neutral 17-ketosteroids in pregnancy, *J. Clin. Endocrinol.* **1**: 142, 1941.
7. Forbes, A. P., and Friedman, E.: Individual variation of 17-ketosteroid excretion. Paper read before 25th Annual Meeting of Association for Study of Internal Secretions, May, 1941.
8. Werner, S. C.: The daily variation in 17-ketosteroid excretion of men and women, *J. Clin. Endocrinol.* **1**: 951, 1941.
9. Talbot, N. B.; Butler, A., and MacLachlan, E.: The colorimetric assay of total alpha and beta 17-ketosteroids in extracts of human urine, *J. Biol. Chem.* **132**: 595, 1940.

10. Heard, R. D. H., and Sobel, H.: A colorimetric method for the estimation of reducing steroids, *J. Biol. Chem.* **165:** 687, 1946.
11. Robbie, W. A., and Gibson, R. B.: Rapid clinical determination of urinary 17-ketosteroids, *J. Clin. Endocrinol.* **3:** 200, 1943.
12. Recant, L.; Forsham, P. H., and Thorn, G. W.: Observations on the pituitary-adrenal response following epinephrine infusion in man, (abstract) *J. Clin. Endocrinol.* **8:** 589, 1948.
13. Goldblatt, H.: Studies on experimental hypertension, *Ann. Int. Med.* **11:** 69, 1937.
14. Page, I. H.: The effect of bilateral adrenalectomy on arterial blood pressure of dogs with experimental hypertension, *Am. J. Physiol.* **122:** 352, 1938.
15. Collins, D. A., and Wood, E.: Experimental renal hypertension and adrenalectomy, *Am. J. Physiol.* **123:** 224, 1938.
16. Goldblatt, H.: The renal origin of hypertension, *Physiol. Rev.* **27:** 120, 1947.
17. McGavack, T. H.: Some pitfalls in the treatment of Addison's disease, *J. Clin. Endocrinol.* **1:** 68, 1941.
18. Soffer, L. J., Engel, F., and Oppenheimer, B.: Treatment of Addison's disease with desoxycorticosterone acetate, *J.A.M.A.* **115:** 1860, 1940.
19. Thorn, G. W.: Treatment of Addison's disease, *J. Clin. Endocrinol.* **1:** 76, 1941.
20. Swingle, W. W.; Parkins, W., and Remington, J.: The effect of desoxycorticosterone and of blood serum transfusions upon the circulation of the adrenalectomized dog, *Am. J. Physiol.* **134:** 503, 1941.
21. Briskin, H. L.; Stokes, F.; Reed, C., and Mrazek, R.: The effects of vitamin D and other sterols on blood pressure in the rat, *Am. J. Physiol.* **138:** 385, 1943.
22. Rodbard, S., and Freed, S. C.: The effect of desoxycorticosterone acetate on the blood pressure of the dog, *Endocrinology* **30:** 365, 1942.
23. Selye, H.: Production of nephrosclerosis in the fowl by sodium chloride, *J. Am. Vet. Med. A.* **103:** 140, 1943.
24. Selye, H., and Hall, C.: Production of nephrosclerosis and cardiac hypertrophy in the rat by desoxycorticosterone acetate overdosage, *Am. Heart J.* **27:** 338, 1944.
25. Selye, H.: Production of hypertension and hyalinosis by desoxocortisone, *Brit. M. J.* **1:** 203, 1950.
26. Rinehart, J. F.; Williams, O., and Cappeller, W.: Adenomatous hyperplasia of the adrenal cortex associated with essential hypertension, *Arch. Path.* **32:** 169, 1941.
27. Sarason, E. L.: Adrenal cortex in systemic disease—a morphologic study, *Arch. Int. Med.* **71:** 702, 1943.
28. Bruger, M.; Rosenkrantz, J., and Lowenstein, B.: Studies on the morphology of the adrenal cortex and on the excretion of 17-ketosteroids in hypertensive patients," *Am. J. M. Sc.* **208:** 212, 1944.
29. Dempsey, W. S.: The adrenal cortex in essential hypertension, *Arch. Path.* **34:** 1031, 1942.
30. Biskind, G. R.: The inactivation of estradiol and estradiol-benzoate in castrate female rats, *Endocrinology* **28:** 894, 1941.
31. Heard, R. D. H., and Hoffman, M. M.: The fate in man of injected alpha estradiol, *J. Biol. Chem.* **141:** 329, 1941.
32. Glass, S. J.; Edmondson, H. A., and Soll, S. N.: Sex hormone changes associated with liver disease, *Endocrinology* **27:** 749, 1940.
33. Girder, H., and Hoagland, C. L.: Urinary excretion of estrogens and 17-ketosteroids in young adult males with infectious hepatitis, *Proc. Soc. Exper. Biol. & Med.* **61:** 62, 1946.

34. Ludewig, S., and Chanutin, A.: Cholesterol studies of adrenals and plasma in partially hepatectomized and partially nephrectomized rats after injection of HN_3, *Endocrinology* **42:** 352, 1948.
35. Zwemer, R. L.: An experimental study of the adrenal cortex, *Am. J. Physiol.* **79:** 658, 1926–27.
36. Bauman, E. J., and Marine, D.: Involution of the adrenal cortex in rats fed with thiouracil, *Endocrinology* **36:** 400, 1945.
37. Tepperman, J.; Engel, F. L., and Long, C. N. H.: A review of adrenal cortical hypertrophy, *Endocrinology* **32:** 373, 1943.
38. Engstrom, W. W., and Mason, H. L.: The excretion of 17-ketosteroids in patients with hyperthyroidism and myxedema, *J. Clin. Endocrinol.* **4:** 517, 1944.
39. Koelsche, G., and Kendall, E. C.: The relation of the suprarenal cortical hormone to nitrogen metabolism in experimental hyperthyroidism, *Am. J. Physiol.* **113:** 335, 1935.
40. Haist, R. E.: Studies in experimental diabetes, *Am. J. Med.* **7:** 585, 1949.
41. Balfour, R. M., and Sprague, R. G.: Association of diabetes mellitus and disorders of the anterior pituitary, thyroid and adrenal cortex, *Am. J. Med.* **7:** 596, 1949.
42. Selye, H., and Collip, J. B.: Fundamental factors in the interpretation of stimuli influencing endocrine glands, *Endocrinology* **20:** 667, 1936.
43. Thorn, G. W.; Forsham, P. H.; Prunty, F. T. G., and Hills, A. G.; A test for adrenal cortical insufficiency, *J.A.M.A.* **137:** 1005, 1948.
44. Heard, R. D. H.; Sobel, H., and Venning, E. H.: The neutral lipide-soluble reducing substances of urine as an index of adrenal cortical function, *J. Biol. Chem.* **165:** 699, 1946.
45. Talbot, N. B.; Butler, A. M.; Berman, R. A.; Rodriguez, P. M., and MacLachlan, E. A.: Excretion of 17-ketosteroids by normal and abnormal children, *Am. J. Dis. Child.* **65:** 364, 1943.

ADRENOGENITAL SYNDROME WITH ASSOCIATED EPISODES OF HYPOGLYCEMIA

FORREST P. WHITE, M.D.* AND LEE E. SUTTON, M.D.
From the Department of Pediatrics, Medical College of Virginia, Richmond, Va.

THE adrenogenital syndrome is a type of hyperadrenocorticism characterized by excess production of adrenal androgens. When it occurs congenitally it is due to hyperplasia of the adrenal glands, and is manifested as pseudohermaphrodism in females and macrogenitosomia praecox in males. Wilkins (1) has recently described the syndrome and collected 99 such cases from the literature and his own observations. A postnatal variety of adrenogenital syndrome also occurs, and is caused usually by adrenal tumors. Forty-one cases are mentioned by Wilkins (1).

Wilkins, Fleischmann, and Howard (2) and Butler, Ross and Talbot (3) in 1939 first described cases of adrenogenital syndrome in which Addisonian symptoms of acute adrenal insufficiency occurred. Zuelzer and Blum (4) recently collected 17 such cases from the literature and added 4 of their own. Since their review, 2 cases have been reported by DeJong and Schaberg (5), 1 case by Chenoweth (6), and 1 case by the New York Hospital group (7). At least 3 and possibly more of 13 cases reported by Allibone, Baar, and Cant (8) as "interrenal syndrome" were probably due to the same condition.

All the reported cases of adrenal insufficiency in adrenogenital syndrome were apparently due to deficiencies, either absolute or relative, of the desoxycorticosterone-like (Na-K) hormone of water and electrolyte metabolism. They were characterized by low serum sodium and high potassium. Blood sugars when recorded were not abnormally low. Determinations of urinary corticosteroids were normal or slightly elevated. There has thus been no clinical or laboratory evidence of deficient production of the adrenal carbohydrate-regulating or "S" hormone.

The following case of the adrenogenital syndrome in a female pseudohermaphrodite is presented because of an associated deficiency of adrenal "S" or carbohydrate-regulating hormone, as evidenced by hypoglycemia. abnormal hypersensitivity to insulin and decreased corticosteroid excretion. In its combination of androgen excess and "S" hormone deficiency, this case is unique.

Received for publication June 30, 1950.

* Present address: 1204 Colonial Avenue, Norfolk 7, Va.

CASE HISTORY

First admission

B. R. a 4-year-old, white, female patient at the Crippled Children's Hospital, had been well except for a quiescent chronic osteomyelitis until February 23, 1950, when she refused breakfast. She was put to bed where she soon vomited greenish-brown liquid and became pale, cool, and unresponsive. She was sent immediately to the Medical College Hospital. A tentative diagnosis of hypoglycemia was made in the Emergency Room and blood was drawn for a sugar determination which was later reported as 20 mg. per cent. During an intravenous infusion of 50 per cent dextrose, the patient suddenly regained consciousness and sat up.

The following history was later obtained from the parents. The child was one of female twins born spontaneously at seven months' gestation, and weighed 3 pounds 2 ounces; the twin weighed 4 pounds. Enlargement of the clitoris was noted at birth. The infant did fairly well on breast milk and in a heated crib at home until the age of 6 weeks, when she suddenly became pale, cold, and limp. Her physician gave her three teaspoonsful of milk and she revived. At 8 weeks, an abscess of the right leg o ırred. The acute inflammation subsided with penicillin therapy, but osteomyelitis developed. Multiple abscesses continued to form until the age of 21 months, and five operations for drainage were performed.

At 21 months of age, the child entered Crippled Children's Hospital and had remained under treatment there, except for visits home. Her development was retarded and she did not sit or crawl until 12 months old. At 24 months, she began hobbling around with her right leg in a cast. Several dark, coarse hairs were noted around the pubis and rectum when the child was 3 years old. While on a visit home in March 1949, the child suddenly vomited before breakfast and became limp, cold, and unconscious. She recovered over a three-day period of treatment with parenteral fluids at a local hospital. At Crippled Children's Hospital in June 1949, following incision and drainage of an abscess from the osteomyelitis, she suffered an unexplained collapse, associated with abdominal distension and acidosis. After a stormy course, she recovered. In August 1949, she was exposed to measles, received an injection of immune globulin, and had a mild case of measles with no sequelae.

Both parents were living and in good health. The mother stated that she had pyelitis and high blood pressure while pregnant with this child. The mother's pregnancies resulted first in a full-term stillborn male; then in 3 normal infants who are now living and well, and finally in the patient and her twin. The twin did well from the first, had always been larger than the patient, and had developed normally both physically and mentally. Among the near relatives, there was no history of any unexplained death in infancy.

Examination of the patient on admission, after she had regained consciousness, revealed a well nourished child who was small for her age, but generally well developed (Fig. 1). The bodily contours were feminine, except that the musculature of the arms and shoulders was unusually well developed. This pronounced development was attributed to the fact that she had used crutches and a walker for months, since the right leg had been in a cast. Her temperature was 98° F., pulse 90, respirations 20, and blood pressure 95/70 mm. Hg.

Her age was $4\frac{1}{12}$ years; weight (with cast), 14.1 Kg.; height, 99 cm.; crown to pubes measurement, 53 cm.; pubes to floor, 46 cm.; span, 93 cm.; chest circumference, 52 cm.; and head circumference 44.5 cm.

The skin was of good turgor and showed no abnormal pigmentation. Tympanic membranes showed scarring but no inflammation. Eyes, nose, and throat were normal. The

chest was clear, and the heart and abdomen were normal. Genitalia: several coarse, dark, long hairs were present around the genitalia and around the anus; the clitoris was definitely enlarged, the prepuce forming a heavy hood-like covering over it; a small vaginal orifice was present in the normal position; no separate urethral opening was seen. Catheterization later revealed that the urethra opened into the vagina, forming a common urogenital sinus. On rectal examination, a small mass was felt which was believed to be the uterus.

Deep reflexes were hyperactive and equal. No pathologic reflexes were noted. The

FIG. 1. **B.R.** Note the enlarged clitoris. Coarse dark hairs present around the pubic area and anus are not visible in this photograph.

child was alert and cheerful. Although she said no understandable words, her response to simple commands and behavior with toys and test materials suggested a mental age of from 18 to 24 months.

Blood studies on admission showed hemoglobin 13.0 Gm., RBC 4.8 million, WBC 21,600 with PMN 61, E 1, L 36, and M 2 per cent. Catheterized urine was normal except for a little traumatic blood.

Other laboratory studies made during hospitalization (February 23, 1950 through April 11, 1950) included: fasting blood sugars, 94, 79, 76, 76, and 84 mg. per cent; NPN 19 mg. per cent; serum bicarbonate 23.0 and 24.5 mEq./L; chloride 103 and 101 mEq./L; sodium 140, 136, and 140 mEq./L; potassium 4.4, 4.3, and 4.9 mEq./L; calcium 11.4 mg. per cent; phosphorus 3.0 mg. per cent; total bilirubin 0.2 mg. per cent;

plasma protein 6.76 Gm. per cent; albumin 3.84 and globulin 2.92 Gm. per cent; BSP retention 6.9 per cent after forty-five minutes. A test with old tuberculin 1:1000 gave negative results in seventy-two hours. There was an eosinophil depression of 43.5 and 56.4 per cent four hours after injection of 0.2 cc. of epinephrine hydrochloride 1:1000. The 24-hour urine volume was 270 cc.; sodium 196 mEq./L; potassium 80 mEq./L; 17-ketosteroids 3.9 mg., and corticosteroids 0.11 mg.

The following 24-hour urine determinations were reported from the laboratory of Dr. Lawson Wilkins: 17-ketosteroids 9.5 mg.; corticosteroids 0.14 mg.; and estrogens (Jailer) 19.9 gamma.

Responses of the fasting blood sugar to glucose, insulin, and epinephrine are shown in Figure 2.

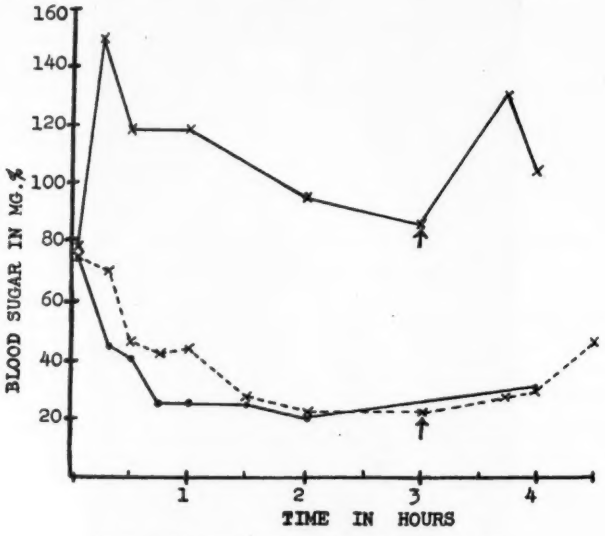


FIG. 2. Glucose and insulin tolerance tests.
X———X Glucose orally; dose, 2 Gm./Kg.
X- - - -X Insulin intravenously; dose, 0.067 U/Kg.
●———● Insulin intravenously; dose, 0.1 U/Kg.

At the points marked by arrows, epinephrine hydrochloride 1–1000 was injected subcutaneously, in a dose of 0.01 cc./Kg.

An electroencephalogram was not entirely satisfactory because the patient was asleep, but no definite abnormality was reported.

X-ray examination of wrists, shoulders, and pelvis suggested a bone age of approximately 6 years. Skull roentgenograms showed moderate convolutional atrophy throughout the skull vault, but no other abnormalities. An intramuscular pyelogram gave normal results.

The child was given a general diet plus a bedtime feeding, but received no extra salt. She did well and had no recurrences of spontaneous hypoglycemia. During the insulin tolerance tests, she became very drowsy and she twitched a few times thirty-five minutes after injection of the insulin (1.5 units intravenously) in the first test. After a period of study, she returned to the Crippled Children's Hospital.

Second admission

The child was readmitted on March 1, 1951, at the age of $5\frac{3}{12}$ years for further studies. Bone age was reported as "at least eight years." Serum calcium was 8.9 mg. per cent, phosphorus 3.9 mg. per cent, and alkaline phosphatase, 3.3 Bodansky units.

In the first 24-hour urine specimen from March 16 to March 17, 1951, the 17-ketosteroids were 10.8 mg., and the neutral reducing lipids, 0.20 mg. The circulating eosinophils were 228/cu. mm. At noon March 19, 1951, 10 mg. of ACTH was started every six hours and continued until noon March 23, 1951. It was planned that the 24-hour urine steroids and the blood eosinophils would be followed during this period and immediately thereafter. Unfortunately, none of the urine specimens was satisfactory after the one reported above. The child was incontinent and an indwelling catheter was inserted. After the first day she became restless and voided frequently around the catheter. The catheter caused irritation so that the urine was contaminated with blood and finally pus. A temperature of 103° F. developed on the afternoon of March 22, 1951, which dropped to normal when penicillin was given and the catheter was removed.

The course of the eosinophil count is shown in Figure 3. The child was discharged March 27, 1951.

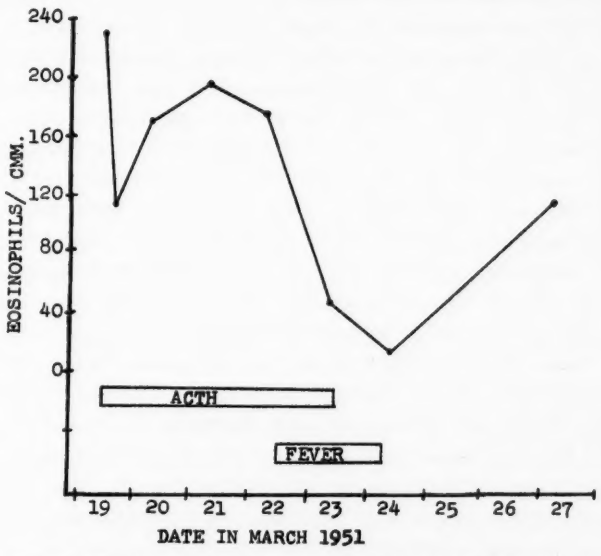


FIG. 3. Response of circulating eosinophils to ACTH, 10 mg. intramuscularly every six hours.

COMMENT

The essential diagnostic criterion in adrenogenital syndrome is excess production of adrenal androgen. This 4-year-old child's 24-hour urinary 17-ketosteroid excretion (3.9 and 9.5 mg.) was at a level appropriate for an adult male. Clinically this was manifested by congenital enlargement of

the clitoris, a common urogenital sinus, and development of coarse dark hairs about the pubic and perianal areas. The bone age was advanced. Muscular development was approximately normal. Excess muscular development is an important feature in the adrenogenital syndrome in the male, but it is not usually seen in the female during early childhood (1). In this case, the preservation of feminine contours may perhaps be explained in part by the moderately increased estrogen production (19.9 gamma in a 24-hour specimen of urine by the Jailer method; normal, 5–10 gamma (9)).

There was no evidence of imbalance in the salt and water-regulating function of the adrenal cortex in this case. Values for serum and urinary electrolytes were repeatedly normal.

The unique feature in this case was the deficiency of carbohydrate-regulating or "S" hormone. Urinary excretion of corticosteroids was low—0.11 and 0.14 mg./twenty-four hours on the first admission, and 0.2 mg. on the second admission (normal range, 0.3 to 0.6 mg.). Clinically, this deficiency of "S" hormone was manifested by the episode of hypoglycemia on admission to this hospital, as well as by three episodes in the past which were probably also due to hypoglycemia. The blood sugar responses to oral glucose and to intravenous insulin and epinephrine are instructive (Fig. 2). After oral administration of 2 grams of glucose per kilogram, the blood sugar rose to 150 mg., then fell rather slowly to normal without any hypoglycemic phase. This type of curve rules out hyperinsulinism as a cause of the patient's hypoglycemia. After the blood sugar had fallen to normal, 0.15 cc. of epinephrine was injected subcutaneously and the level rose again to 130 mg. This suggests that there is no significant impairment of hepatic glycogenolysis in response to epinephrine.

Insulin tolerance was measured on two occasions, using insulin intravenously in dosages of 0.1 U/Kg. and 0.067 U/Kg. The blood sugar dropped to levels of 20 mg. and 22 mg. In each instance the minimum level was not reached for two hours, and the recovery phase then began so slowly that the levels were still 30 mg. at four hours (Fig. 2). This slowness with which the minimum levels were reached is probably explainable by the sustaining effect which hepatic glycogenolysis had upon the blood sugar level. Exhaustion of hepatic glycogen—which was demonstrated by the lack of response to epinephrine injected at the three-hour mark in one of the tests—removed this sustaining effect and allowed severe hypoglycemia to occur. In the normal person, the blood sugar is also sustained and severe hypoglycemia prevented by gluconeogenesis of glucose from amino acids under the influence of adrenal "S" hormone. Thus, the extreme hypersensitivity to insulin of our patient is strong evidence of a deficiency in her production of the adrenal "S" hormone of gluconeogenesis.

The eosinophil depression after epinephrine injection, averaging 50.0 per cent on two occasions, is well within the normal limits for children, as previously described by us (10). On the second admission the response to repeated injections of ACTH in a dose of 10 mg. every six hours, was determined (Fig. 3). Eosinophil depression after the first dose was again normal (49 per cent), but during the next three days the eosinophil level became stabilized at about 15 to 25 per cent below the initial level. Normally a progressive fall to levels near zero would be expected. Since eosinophil depression is affected by the carbohydrate-regulating hormones, this is further evidence that this patient's ability to produce these hormones was severely limited and quickly exhausted. Of interest is the fact that the eosinophil level dropped to 11/cu. mm. the day after ACTH was stopped. This decrease appeared to be a response to the urinary infection and fever, which occurred at that time.

The finding of low serum phosphorus (3.0 mg. on the first admission, and 3.9 mg. on the second admission) is of interest. We were unable to determine whether this was caused by the chronic osteomyelitis, by the altered carbohydrate metabolism, or by the increased androgen production. It is quite possible that all three factors had their effect.

In brief, the production of carbohydrate-regulating hormone was apparently adequate for the patient's usual needs, as manifested by the normal blood sugar values, normal hepatic glycogenolysis in response to epinephrine and ACTH, and only moderate lowering of the 24-hour excretion of corticosteroids. That this hormone was quickly exhausted and inadequate for stress is indicated by the episodes of hypoglycemia, the prolonged shock after minor surgery, hypersensitivity to insulin, and abnormal eosinophil response to repeated injection of ACTH.

SUMMARY

A case history is presented of the adrenogenital syndrome in a 4-year-old, white female pseudohermaphrodite, who showed clinical and laboratory evidence of adrenal insufficiency with respect to the carbohydrate-regulating or "S" hormone. To our knowledge, this combination of excess adrenal androgen and deficient adrenal "S" hormone has not previously been reported.

Acknowledgment

We are indebted to Drs. H. St. George Tucker and Isabel Taliaferro of the Department of Endocrinology and to Dr. Lawson Wilkins of Johns Hopkins Hospital for their advice and for many of the adrenal steroid determinations.

REFERENCES

1. Wilkins, L.: Hyperadrenocorticism, *Pediatrics* **3**: 533, 1949.

2. WILKINS, L.; FLEISCHMANN, W., and HOWARD J. E.: Macrogenitosomia precox associated with hyperplasia of the androgenic tissue of the adrenal and death from corticoadrenal insufficiency; a case report, *Endocrinology* **26:** 385, 1940.
3. BUTLER, A. M.; ROSS, R. A., and TALBOT, N. B.: Probable adrenal insufficiency in an infant, *J. Pediat.* **15:** 831, 1939.
4. ZUELZER, W. W., and BLUM, A., JR.: Adrenocortical insufficiency in infants with the adrenogenital syndrome, *J. Pediat.* **35:** 344, 1949.
5. DEJONG, S. J., and SCHABERG, A.: The adrenogenital syndrome in the newborn, *Maandschr. v. Kindergeneesk.* **15:** 392, 1947.
6. CHENOWETH, B. M., JR.: Dysfunction of the adrenal gland in infancy, *Southern M. J.* **41:** 307, 1948.
7. New York Hospital—Cornell Medical Center: Clinical Conference, *J. Pediat.* **34:** 768, 1949.
8. ALLIBONE, E. C.; BAAR, H. S., and CANT, W. H. P.: The interrenal syndrome in childhood, *Arch. Dis. Child.* **22:** 210, 1947.
9. WILKINS, L.: Personal communication, 1950.
10. WHITE, F. P., and SUTTON, L. E., JR.: Observations on pituitary-adrenal response to epinephrine, *Pediat.* **5:** 876, 1950.
11. RECANT, L.; FORSHAM, P. H., and THORN, G. W.: Observations on the pituitary-adrenal response following epinephrine infusion in man, (abstract) *J. Clin. Endocrinol.* **8:** 589, 1948.
12. LARAGH, J. H., and ALMY, T. P.: Eosinopenia after epinephrine, insulin, and surgery, *Proc. Soc. Exper. Biol. & Med.* **69:** 499, 1948.
13. THORN, G. W.; FORSHAM, P. H.; PRUNTY, F. T. G., and HILLS, A. G.: A test for adrenal cortical insufficiency, *J.A.M.A.* **137:** 1005, 1948.

LETTERS TO THE EDITOR

HORMONAL ACTIVITY IN SEMEN

To the Editor:

The search for testicular hormones or principles capable of causing inhibition of pituitary gonadotropin production led us to investigate the effect of parenteral administration of semen to male and female rats. At this time we were unaware of the findings of Dirscherl *et al.* (1), Green-Armytage *et al.* (2) and Riisfeldt (3). The last author, using subcutaneous injections of whole semen, produced estrus in spayed rats and hemorrhagic follicles in immature animals. Dirscherl, using a color reaction which he feels to be characteristic of substances of the trans-dehydroandrosterone group, found this reaction to be positive with extracts of human and bull spermatozoa. Green-Armytage *et al.*, working on the hypothesis that there must be present in semen a principle capable of causing the enlargement of the uterus observed in women after a few months of marriage, found uterine stimulation in immature rats, mice and does, using injections of whole semen. Extracts of the precipitate formed after addition of acetone were also found to be active by these investigators. Estrogenic activity has, so far as we are aware, never been related to the spermatozoa.

Our own investigations have comprised the use of human and bull saline suspensions of spermatozoa and later on, of ether extracts of the washed cells. Table 1 shows the effects obtained with such injections in the immature female rat and in the spayed adult rat sacrificed five days after the daily administration of the preparations. Uteri and ovaries were weighed fresh, but adrenals and thymus were weighed after fixation in Zenker's fluid. The uteri were filled with fluid and some of the injected animals' ovaries showed hyperemia. The vaginas were closed in all immature animals and vaginal smears in the adult spayed animals showed no estrus. Evidence of toxicity was apparent in the ether extract by the adrenal enlargement and thymus involution present in the treated animals (Exp. No. 2). Assay of single human ejaculates in saline suspensions have so far indicated no direct correlation between the uterine weight stimulation and the sperm cell count. One of such experiments is recorded in Table 1 (Exp. No. 3).

Similar assays have been performed in the immature castrate male rat using the prostatic phosphatase method (Schaffenburg and McCullagh, (4)) as a measure of androgenic activity. The results of some of these ex-

periments are recorded in Table 2. Extracts were prepared by the method of Friedgood and Garst (5) for the fractionation of phenolic and neutral steroids after hydrolysis of the washed spermatozoa with concentrated HCl. The neutral fraction was used after dissolving it in sesame oil. Injections were given daily for three days and the animals were sacrificed on the fifth day. Prostatic alkaline phosphatase was determined by the method of Huggins and Talalay (6).

TABLE 1. EFFECT OF INJECTIONS OF SPERMATOZOA IN THE FEMALE RAT

Exp. No.	Description	Treatment of spermatozoa	Assay animals	Weight (mg.)			
				Uterus	Ovaries	Adrenals	Thymus
1	Pooled human semen, 50 cc. Count: 46 mill./cc.	Saline cell-suspensions	5 immat. ♀ rats	67.4	27.3	—	—
	Controls		5 immat. ♀ rats	30.5	23.0	—	—
2	Pooled bull semen, 27 cc. Count: 4 bill./cc.	Ether ext. of unhydrol. cells in oil solution	4 adult ♀ spayed rats	119.7	—	35.7	199.1
	Controls		3 adult ♀ spayed rats	65.0	—	30.1	238.6
3	Human semen						
	A. 3.0 cc. 15 mill./cc.	Saline cell-suspensions	1 immat. ♀ rat	20.4	19.2	12.8	150.6
	B. 2.8 cc. 5 mill./cc.		1 immat. ♀ rat	19.2	22.0	10.0	98.2
	C. 3.0 cc. 6 mill./cc.		1 immat. ♀ rat	22.0	24.6	12.0	162.6
	D. 3.0 cc. 5 mill./cc.		1 immat. ♀ rat	24.6	23.6	13.0	121.0
	Pooled bull semen, 13 cc. Count: 530 mill./cc.	Saline cell-suspensions	4 immat. ♀ rats	28.3	25.1	11.6	84.2
	Controls		4 immat. ♀ rats	16.7	21.0	9.8	130.1

TABLE 2. EFFECT OF EXTRACTS OF SPERMATOZOA IN THE IMMATURE CASTRATE MALE RAT

Description	Treatment of spermatozoa	No. of rats	Prostatic alk. phosphatase content (units)
Pooled human semen, 21.1 cc. Count: 67.2 mill./cc.	HCl-hydrolyzed ext. of cells; neutral fract. dissolved in oil	3	17.1
Controls	Uninjected	6	10.5
Testosterone controls	500 μg. Testosterone propionate	6	34.2

Assay of saline cell-suspensions as well as of ether extracts of unhydrolyzed specimens showed little or no activity.

We believe that these preliminary experiments strongly indicate the presence of androgenic and estrogenic activity in spermatozoa. The fact that saline cell-suspensions and hydrolyzed extracts are, for the most part, active, whereas the product of direct ether extraction of the cells without previous hydrolysis shows little activity, seems to indicate that the steroids present are in a conjugated form. We should like to point out, in passing, the extreme toxicity of some of the ether extracts of unhydrolyzed, washed cells. Subcutaneous injections of this material dissolved in oil into immature rats caused profuse capillary hemorrhages with hemolysis and some necrosis spreading widely around the tissues adjacent to the site of injection. The animals died shortly after and the collecting tubules of the kidney were found to be full of pigment casts (myoglobin ?) and red cells. Precipitation of "phospholipid" with saturated magnesium chloride after solution in acetone did not remove toxicity. It was, however, removed after extraction with alkali.

Investigations carried on at present are directed toward the chemical identification of the substances responsible for the hormonal activity, their possible role in the problem of fertility and the nature of the toxic "hemolytic" principle present in sperm cells.

From the Cleveland Clinic and the Frank E. Bunts Educational Institute, Cleveland, Ohio.

E. Perry McCullagh, M.D.
C. A. Schaffenburg, M.D.

Acknowledgments

This investigation has been supported by a grant from Mr. Dean Francis.
The technical assistance of Miss D. Tweed is gratefully acknowledged.

REFERENCES

1. Dirscherl, W., and Knüchel, W.: Über das Vorkommen von Androgenen im menschlichen und tierischen Sperma., *Biochem. Ztschr.* **320:** 253, 1950.
2. Green-Armytage, V. B.; Silberstein, F., and Wachtel, G. E.: The influence of semen on the female reproductive organs, *J. Obst. & Gynaec., Brit. Emp.* **54:** 324, 1947.
3. Riisfeldt, O.: Demonstration of substances with estrogenic effect in human sperm, *Acta endocrinol.*, **1:** 217, 1948.
4. Schaffenburg, C. A., and McCullagh, E. P.: Specific androgen stimulation of the rat's prostatic phosphatases as a basis for the assay of androgenic material, *J. Lab. & Clin. Med.* **36:** 983, 1950.

5. FRIEDGOOD, H. B., and GARST, J. B.: Identification and quantitative microdetermination of estrogens by ultraviolet absorption spectrophotometry, *Recent Progr. Hormone Research* **2**: 31, 1948.
6. HUGGINS, C., and TALALAY, P.: Sodium phenolphthalein phosphate as a substrate for phosphatase tests, *J. Biol. Chem.* **159**: 399, 1945.

EFFECT OF IODOTHIOURACIL ON THE SERUM PROTEIN-BOUND IODINE

TO THE EDITOR:

The experimental evidence of the antithyroid activity of iodothiouracil has led to the introduction of this compound into clinical practice for the treatment of hyperthyroidism. In our preliminary tests of the drug, we were disturbed to discover that the material is carried down during the precipitation of proteins for the determination of the serum protein-bound iodine. Consequently, the administration of iodothiouracil produces a very pronounced rise in the PBI. The administration of 50 mg. of iodothiouracil four times daily to 5 normal subjects uniformly produced an elevation of the PBI to levels too high to read (more than 26 micrograms per 100 ml.) within twenty-four hours. When the serum was diluted, it was estimated that the PBI concentration ranged from 63 to 119 micrograms per 100 ml.

The use of the PBI determination is becoming increasingly widespread in clinical practice. We have found it to be a valuable aid in following the treatment of patients with hyperthyroidism. The fact that iodothiouracil interferes with the determination appears to lessen the value of the drug. This is particularly true, since other medications which do not have this effect are available. In any case, it seems worth while to emphasize the fact that the administration of iodothiouracil can be expected to invalidate any further use of the serum protein-bound iodine determination until some time after the medication has been discontinued.

PHILIP K. BONDY, M.D.

Grady Memorial Hospital,
Atlanta, Georgia.

The Association for the Study of Internal Secretions

THE 1952 ANNUAL MEETING

The Thirty-fourth Annual Meeting of The Association for the Study of Internal Secretions will be held at the Palmer House, Chicago, Illinois, Thursday, Friday, and Saturday, June 5–6–7, 1952.

The Local Chairman of Arrangements is Dr. Willard O. Thompson.

All Scientific Sessions will be held in the Palmer House. The rooms in which each session will be held will be announced in the program and on the hotel bulletin board. The Annual Dinner is scheduled for Friday, June 7, at 7:30 P.M., preceded by cocktails at 6:30 P.M.

All members are urged to make hotel reservations immediately, inasmuch as the hotels expect to be filled to capacity. Make reservations directly with the Palmer House, advising time of arrival and departure date. Make your reservations now and avoid disappointment.

Those wishing to present papers, which will be limited to ten minutes, should send four copies of the title and abstract to the Vice-President, Dr. Evelyn Anderson, National Institutes of Health, Bethesda 14, Maryland, not later than *March 1, 1952. It is imperative that the abstracts be informative and complete with results and conclusions in order that they may be of reference value and suitable for printing in the program and journals of the Association.* The following regulations for the preparation of abstracts and titles must be carefully followed to insure consideration of the paper for the program:

1. Abstracts may not exceed two hundred words, or equivalent space, exclusive of title. No footnotes or acknowledgments to sponsors can be published. References, if used, must be placed in the body of the text. The abstract should consist of a single paragraph, if possible. Structural chemical formulas cannot be used.
2. The title heading must be arranged as follows:
   Line 1. Title, not to exceed fifteen words.
   Line 2. Author/s. The name of each nonmember-author collaborating with member-authors is to be followed by the phrase

"(by invitation)." Names of nonmembers who are introduced, *i.e.*, who are not collaborators with member-authors, are to be followed by the phrase "(introduced by . . .)." The principal degree, *e.g.*, M.D., of each author should be given after his name.

Line 3. Institution of origin and city in which institution is located.

3. The body of the abstract, typed double-space, should follow the heading. The original copy should be on bond paper. There should be three copies.
4. Abstracts should be letter-perfect, since there will be no opportunity for proofreading by authors.

1952 AWARDS AND FELLOWSHIPS

Fellowships

THE SCHERING FELLOWSHIP IN ENDOCRINOLOGY

This Fellowship will not be awarded in 1952, so that it may accumulate toward a $5,000 Fellowship to be awarded in 1953.

THE AYERST, McKENNA AND HARRISON FELLOWSHIP

The Ayerst, McKenna and Harrison Fellowship was first awarded in 1947 to Dr. Samuel Dvoskin and in 1948 to Dr. Ernest M. Brown, Jr. Dr. Brown was re-elected for the Fellowship in 1949. The 1950 Award was made to Dr. Lawrence E. Shulman.

Association Fellowships are designed to assist men or women of exceptional promise in their progress toward a scientific career in endocrinology. Fellowships may be awarded to an individual who possesses the Ph.D. or M.D. degree or to a candidate for either of these degrees. The stipend, which will not exceed $5,000, may be divided into two Fellowships in varying amounts in accordance with the qualifications of the appointee. The Committee will, in reviewing the proposed program of study, consider the amount of time which the Fellow intends to spend in course work and/or teaching. He must present evidence of scientific ability as attested by studies completed or in progress and/or the recommendation of responsible individuals. He must submit a program of proposed study, indicate one or more institutions where the proposed program will be followed, and submit statements of approval from the investigators with whom he proposes to conduct his research. He must serve full time if awarded a Fellowship. A small amount of time (10 to 15 per cent) may be allotted for course work or for participation in teaching, the latter purely on a voluntary basis.

Awards

THE E. R. SQUIBB AND SONS AWARD

The E. R. Squibb & Sons Award of $1,000 was established in 1939, and was given first in 1940 to Dr. George W. Corner; in 1941 to Dr. Philip E. Smith; in 1942 to Dr. Fred C. Koch; in 1944 to Dr. E. A. Doisy; in 1945 to Dr. E. C. Kendall; in 1946 to Dr. Carl G. Hartman; in 1947 to Drs. Carl F. and Gerty T. Cori; in 1948 to Dr. Fuller Albright; in 1949 to Dr. Herbert M. Evans; in 1950 to Dr. C. N. H. Long; and in 1951 to Dr. J. B. Collip. In 1943 no award was given. A special committee of five members of the Association selects the recipient from among investigators in the United States or Canada, on the basis of outstanding contributions to endocrinology.

THE CIBA AWARD

The Ciba Award, to recognize the meritorious accomplishment of an investigator not more than thirty-five years of age in the field of clinical or pre-clinical endocrinology, was established in 1942, but no recipient was selected in 1942 or 1943. In 1944 the award was presented to Dr. E. B. Astwood; in 1945 to Dr. Jane A. Russell; in 1946 to Dr. Martin M. Hoffman; in 1947 to Dr. Choh Hao Li; in 1948 to Dr. Carl Heller; in 1949 to Dr. George Sayers; in 1950 to Dr. Oscar M. Hechter; and in 1951 to Dr. Albert Segaloff. The Award is for $1,200. If, within twenty-four months of the date of the Award, the recipient should choose to use it toward further study in a laboratory other than that in which he is at present working, it will be increased to $1,800.

Nominations

Each member has the privilege of making one nomination for each Fellowship or Award. A nomination should be accompanied by a statement of the importance of the nominee's contributions to, or interest in, endocrinology and by a bibliography of the nominee's most important publications, with reprints if possible. The nominations should be made on special application forms which may be obtained from the Secretary, Dr. Henry H. Turner, 1200 North Walker Street, Oklahoma City 3, Oklahoma, and returned to him *not later than March 1, 1952.*

The American Goiter Association

THE 1952 ANNUAL MEETING

The next Annual Meeting of the American Goiter Association will be held in St. Louis, Missouri, May 1, 2 and 3, 1952, at the Hotel Chase. All those who plan to attend are urged to make hotel reservations immediately.

VAN METER PRIZE AWARD FOR 1952

The American Goiter Association again offers the Van Meter Prize Award of three hundred dollars and two Honorable Mentions for the best essays submitted concerning original work on problems related to the thyroid gland. Providing essays of sufficient merit are presented in competition, the Award will be made at the annual meeting of the Association which will be held in Saint Louis, Missouri, May 1, 2 and 3, 1952.

The competing essays may cover either clinical or research investigations; should not exceed three thousand words in length; must be presented in English; and a typewritten double-spaced copy in duplicate sent to the Corresponding Secretary, Dr. George C. Shivers, 100 East Saint Vrain Street, Colorado Springs, Colorado, *not later than March 1, 1952*. The committee, who will review the manuscripts, is composed of men well qualified to judge the merits of the competing essays.

A place will be reserved on the program of the annual meeting for presentation of the Prize Award Essay by the author, if it is possible for him to attend. The essay will be published in the annual Transactions of the Association.

* * *

AWARD FOR OUTSTANDING RESEARCH IN THE FIELD OF INFERTILITY

The American Society for the Study of Sterility announces the opening of the 1952 contest for the most outstanding contribution to the subject of infertility and sterility. The winner will receive a cash award of one thousand dollars, and the essay will appear on the program of the 1952 meeting of the Society. Essays submitted in this competition must be received not later than *March 1, 1952*. For full particulars concerning requirements of this competition, address The American Society for the Study of Sterility, 20 Magnolia Terrace, Springfield, Massachusetts.